W-AM-1S THEORETICAL STUDIES OF PROTEIN DYNAMICS. M. Karplus, Department of Chemistry, Harvard University, Cambridge, Mass. 02138

The internal motions occuring in proteins will be described and theoretical approaches to their calculation will be outlined. It will be shown how empirical energy functions, when used with transition state, vibrational analysis, and molecular dynamics techniques can be employed to obtain a detailed picture of the possible motions over a wide range of characteristic times. Examples taken from studies of the pancreatic trypsin inhibitor, myoglobin, and lysozyme, will be presented.

W-AM-2S NUCLEAR MAGNETIC RESONANCE PROBES OF INTERNAL MOTION IN PROTEINS. Brian D. Sykes. Department Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

Nuclear magnetic resonance spectra and relaxation time measurements are sensitive probes of internal motions in proteins, such as segmental motion of amino acid side chains and conformational changes. The usefulness of various nuclei such as intrinsic ^{1}H or ^{13}C, or ^{19}F introduced as a label, will be discussed along with an analysis of the time scales accessible to NMR. Examples from current results will be given including studies of the motion of individual tyrosines in the bovine pancreatic trypsin inhibitor and individual fluorotyrosines in a fluorotyrosine alkaline phosphatase prepared from E. coli.

W-AM-3S EPR AND FLUORESCENCE PROBING OF LARGE-SCALE ROTATIONAL MOVEMENTS. David D. Thomas, Dept. of Muscle Research, Boston Biomedical Research Inst., 20 Staniford St., Boston, MA 02114

In recent years there have been dramatic advances in obtaining biochemical and structural information about large biological molecules and about large, organized supramolecular assemblies such as membranes, muscle fibers, and chromosomes. As a result, increasing interest has developed in spectroscopic methods that can be used to detect the large-scale, restricted, and relatively slow motions (characterized by times in the range of tens of nanoseconds to milliseconds) that are expected for large segments of molecules and for entire molecules within supramolecular assemblies. Two methods that have proven most effective in studying rotational motions in the nanosecond-to-millisecond range are electron paramagnetic resonance (EPR), usually involving the introduction of nitroxide spin labels, and fluorescence depolarization, involving both intrinsic and extrinsic fluorophores. Until recently these methods were usually limited to the study of rotational correlation times on the order of a microsecond or less, and were used to study the tumbling of isolated proteins in solution and segmental motions within proteins. Fluorescence has been especially powerful for such studies, as exemplified by the work on internal motions in antibodies (Yguerabide, Epstein, and Stryer) and in myosin (Mendelson, Morales, et al). The development of fluorescent probes with longer lifetimes and the development of saturation transfer EPR have extended the sensitivities of both methods to correlation times much longer than a microsecond. My colleagues and I have used saturation transfer EPR to measure quantitatively the rotational correlation times in the range of 10^{-7} to 10^{-3} sec for myosin heads (cross-bridges) and actin molecules in model muscle systems. We have also used saturation transfer spectroscopy to study the slow rotational motion of a membrane protein, the Ca^{2+}-ATPase of sarcoplasmic reticulum. (D.D.T. is a Research Fellow of MDAA)

W-AM-4S KINETIC STUDIES OF PROTON EXCHANGE AND OF CONFORMATIONAL REACTIONS. R.L. Baldwin
Department of Biochemistry, Stanford University, Stanford, CA. 94305

The functioning of some proteins apparently requires the participation of rare, partly open, conformational states. An example, illustrated by Englander's work, is the problem of how O_2 gains access to the heme iron of myoglobin and hemoglobin. The existence of partly open conformational states is shown most clearly by proton exchange studies. Progress in analyzing the structural mechanisms of opening has been slow because of difficulties in obtaining exchange rates for individual protons at known locations in the protein's structure. However, high resolution proton NMR measurements are beginning to provide these data. Work from the groups of Sykes and Wüthrich has shown that the lines of individual amide protons are resolved for the small protein BPTI (trypsin inhibitor) in D_2O; changes in line intensities yield exchange rates for individual protons. Lanthanide shift experiments have been used to partially assign some of these protons to the β-pleated sheet of BPTI. The folded structure of BPTI provides a high degree of protection against exchange for about eleven amide protons. The data suggest that the exchange behavior of each proton is unique, each with its own dependence on pH and temperature. A resolution enhancement technique has been used by Patel and Canuel to resolve many amide protons in a larger protein, ferrocytochrome c, and data for exchange rates have been obtained. A conventional ^3H exchange technique has been used by Schreier to study the exchange behavior of a specific moiety of a dissociable protein, the S-peptide of ribonuclease S. The most highly protected protons (about six) exchange at a fairly uniform rate; these protons are probably in the H-bonded α helix of residues 7-13. Comparison between dissociation of the S-peptide and opening to permit exchange in the bound state shows that the two reactions have similar properties as regards dependence on pH, temperature, and ionic strength.

W-AM-A1 VOLTAGE CLAMP EXPERIMENTS ON NORMAL & DENERVATED RAT SKELETAL MUSCLE FIBERS. P. Pappone* (Intro. by A. M. Gordon), Dept. Physiol., U. of Wash., Seattle, WA 98195.

Sodium currents in voltage-clamped single fibers from rat sternomastoid (SM) and EDL muscles were studied at 12°C using the vaseline gap method of Hille and Campbell (JGP 67:265, 1976). Large fibers were selected; when mounted in the vaseline gap and the ends cut in 160 mM CsF the fibers contracted and their diameter increased to 80-120 μm. Average resting membrane resistance was about 300 Ωcm^2 (SM) or 570 Ωcm^2 (EDL), average membrane capacity was about 11 $\mu F/cm^2$ (SM). Peak sodium conductance averaged 41 (±19 S.D., EDL) and 150 (±56 S.D.) $mmho/cm^2$ (SM). The kinetics of the currents at 12°C were similar to those found by Hille and Campbell in frog muscle at 5°C assuming a Q_{10} of 3. The permeability of several organic and inorganic cations relative to sodium was obtained from the change in sodium channel reversal potential after replacing all external Na^+ with the test ion. The permeability ratios, P_{ion}/P_{Na}, in SM fibers were: Na 1.00; Li 0.985; hydroxylammonium 0.996; hydrazinium 0.274; guanidinium 0.192; ammonium 0.167; and K^+ 0.051. Sodium currents in 5-day denervated EDL fibers were investigated. Their kinetics were essentially the same as those of innervated fibers, although the denervated fibers showed more variability. Denervated fibers were more resistant than the innervated fibers to block by TTX. While the dose-response curve of innervated fibers was consistent with a single K_{diss} for TTX of 3.16 nM, that of denervated fibers suggested the presence of two populations of sodium channels. The results in denervated fibers could be fit by assuming that 80% of the peak conductance was blocked by TTX with a K_{diss} of 9.7 nM, and the remainder was blocked with a K_{diss} in the micromolar range. (Supported by USPHS grants AM-17803, GM-00260, NS-08174)

W-AM-A2 EFFECT OF NaCN ON FROG ATRIAL MUSCLE: A CURRENT AND VOLTAGE CLAMP STUDY. J.Nargeot*, C.E. Challice, K-S Tan* and D. Garnier*, Department of Physics, University of Calgary, Calgary T2N 1N4, Canada, and Laboratoire de Physiologie Animale, Faculté des Sciences, Parc de Grandmont, 37200, Tours, France.

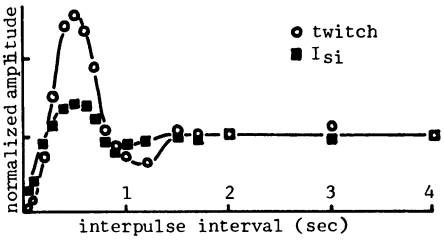
Metabolic inhibitors (e.g. 2,4 Dinitrophenol and cyanide) and anoxia both induce a shortening of heart action potentials accompanied by a decrease in the amplitude of contraction. By comparing the observations in the two cases, it was hoped to obtain new information on the mechanism of metabolic inhibition. Current and voltage clamp studies were carried out on frog atrial fibers in 5×10^{-3} M NaCN at pH 7.6, by the double sucrose gap technique, with contraction recorded by a needle in the central compartment connected to a transducer. After 10 to 15 minutes of NaCN treatment there was (1) a reduction in both the action potential duration and plateau amplitude along with a reduction in the contractile force· (2) in Ringer TTX a marked reduction in the magnitude of slow inward current and corresponding tension; (3) a negative shift of the reversal potential of the slow inward current. Introduction of excess Ca^{++} did not increase the slow inward current amplitude although the I-V relationship showed a resotration of the Ca^{++} driving force, indicating that the slow channel conductance is also affected during NaCN treatment. However, the application of 10^{-7} M Adrenaline during poisoning induced a rapid increase in slow inward current and phasic contraction amplitude. It is thus concluded that NaCN reduces the slow inward current and corresponding tension, decreasing both the Ca^{++} driving force and slow channel conductance. The strong increase in the slow inward current and the phasic contraction with Adrenaline suggest that high energy compounds such as cyclic AMP would be present in spite of the metabolic inhibition and would prevent the increase of internal free Ca^{++} concentration and also increase the slow channel conductance.

W-AM-A3 ROLES OF CALCIUM AND SODIUM IONS IN THE TRANSIENT INWARD CURRENT INDUCED BY STROPHANTHIDIN IN CARDIAC PURKINJE FIBERS. R. Weingart*, R. S. Kass and R. W. Tsien, Department of Physiology, Yale University School of Medicine, New Haven Connecticut 06510.

Digitalis intoxication promotes arrhythmogenic spontaneous activity in Purkinje fibers by inducing a novel transient inward current (TI). The TI appears as an inward hump which peaks about 1 sec following the break of a strong depolarizing pulse. Participation of Ca ions has been suggested because the TI is sensitive to Ca_o, Mn or D600, and can be accompanied by a phasic aftercontraction (AC) with similar waveform. The previous results allow two alternate interpretations. The TI could be a transient Ca influx, or it could be explained as a phasic release of Ca from an internal store, that not only activates contraction but also regulates ionic current across the surface membrane. Our voltage clamp experiments favor the second hypothesis. The AC can sometimes be observed in the absence of the TI. Furthermore, the TI has a reversal potential near 0 mV that can be demonstrated by repolarizing from +25 mV to various levels. The inverted TI is easily identified because its waveform remains similar to the AC. Since E_{rev} is far from E_{Ca} and not changed by doubling Ca_o, Ca ions cannot be the major charge carrier. As sodium is replaced with choline or Tris, the reversal potential shifts to more negative values and levels off at -25 mV. This suggests that Na influx contributes to the TI. The pathway for the Na movement is probably not the excitatory Na channel because TTX abolishes excitability without inhibiting the TI. Since $E_{rev} \neq E_{Na}$, the current cannot be specific for Na. K efflux may be involved since Cl replacement argues against Cl as a carrier of outward TI current. This leaves 'background' or 'leak' channels for Na and/or K ions as possible pathways. Ca_i may activate such channels and thereby control the TI time course. Oscillatory changes in Ca_i could also explain our finding that periodic fluctuations of tension and current occur in strophanthidin and have similar power spectra.

W-AM-A4 STROPHANTHIDIN CAN INCREASE THE SLOW INWARD CURRENT AND ALTER ITS REPRIMING KINETICS. R.S. Kass, R.W. Tsien and R. Weingart*, Department of Physiology, Yale University School of Medicine, New Haven Connecticut 06510.

Cardiotonic steroids increase the strength of the cardiac contraction. They also alter the restitution of the ability to contract following a conditioning beat, changing the time course of recovery from an exponential to an oscillatory curve. We find that both of these effects correlate with changes in the slow inward current (I_{si}). Tension and membrane current are measured in short calf Purkinje fibers. A two microelectrode voltage clamp is imposed for brief periods which periodically interrupt ongoing beats. Strophanthidin (1 μM) produces rapidly developing but reversible effects. It increases twitch tension, elevates the plateau and augments I_{si}. Increases in I_{si} have already been seen in frog atrial muscle by Brown, Giles and S.J. Noble (unpublished) who found definite but variable effects with ouabain. Thus, at an early stage of drug action, increases in I_{si} may contribute to or reflect the positive inotropic effect. (Later on, tension continues to increase but I_{si} declines, suggesting the involvement of other mechanisms). Strophanthidin also influences the restitution of contraction and recovery of I_{si} in two-pulse experiments. I_{si} was dissected with D600. Twitches and I_{si} showed rather similar dependence on the interval between pulses (Figure). The oscillatory recovery of I_{si} in strophanthidin suggests that previous Hodgkin-Huxley type descriptions of I_{si} may need modification.



W-AM-A5 MEMBRANE ELECTRICAL PROPERTIES OF FROG SLOW MUSCLE FIBERS. C.S. Hui* and W.F. Gilly* (Intr. by C.L. Slayman), Dept. Physiology, Yale Univ. Medical School, New Haven, CT.

Little is known concerning properties of membrane capacitance and ionic conductances in frog slow fibers, and we have begun studying these questions using the 3-microelectrode voltage clamp method (Adrian et al, 1970, J. Physiol. 208,607) on pyriformis slow fibers of Rana temporaria at 5-8°C. Fibers in isotonic Ringer have a membrane capacitance referred to cylindrical fiber area of 2-3 $\mu F/cm^2$ (50-60 μ dia.), while those in a paralyzing solution of D_2O Ringer + 230mM sucrose give 4-5 $\mu F/cm^2$ (60-70 μ dia. in the hypertonic Ringer). At the holding potential of -90 mv, resting conductance is largely leakage at the impalement sites, and ionic conductance is no more than 5-10 $\mu mho/cm^2$. Depolarizations over the potential range of -60 to 0 mv progressively activate a conductance producing outward currents with a sigmoid time course resembling the delayed K^+ currents in frog twitch fibers. Maximum slope conductance taken from the "steady-state" current-voltage relation is about 350 $\mu mho/cm^2$. A second slower rise in outward current appears with depolarizations past -30 mv or so. Large outward currents are well maintained during long (5-10 sec) depolarizations to membrane potentials as high as +40 mv. No net inward currents have been observed.

W-AM-A6 DEPOLARIZING AFTERPOTENTIAL IN CRAB MUSCLE FIBERS. A.L. Sorenson* and G. Suarez-Kurtz* (Intr. by J. Wittenberg). Dept. Farmacologia, UFRJ, Rio de Janeiro, Brazil.

Isolated muscle fibers of Callinectes danae respond to depolarizing current with rapid, transient Ca-dependent electrogenesis. A prominent depolarizing afterpotential (DAP) occurs after termination of the applied current; it consists of a small (5-10 mV) transient depolarization lasting from 50-100 msec. Both the rapid and the after depolarization are graded with stimulus strength. The ionic basis of the DAP however appears to differ from that of the initial rapid event. Although tetrodotoxin has no effect, replacement of Na by Li or Tris abolishes the DAP while the initial Ca-dependent depolarization remains. Lowered Ca or Mg on the other hand enhances the DAP while elevated Ca or Mg depresses it. Altering K has little effect on the magnitude but the DAP is terminated more rapidly in elevated K. The DAP is associated with a decrease in effective membrane resistance which is greatest in the early stages and which gradually returns to normal values as repolarization occurs. Our results suggest that the DAP in crab muscle fibers results from an inward Na current. There appears to be no direct relation of the afterpotential to contraction however, for although the DAP has a similar time course to that of contraction, contraction persists in the absence of the DAP following removal of Na.

Research support from the CNPq, Brasil.

W-AM-A7 USE OF FLUORESCENT DYES TO EVALUATE SINGLE SUCROSE GAP VOLTAGE CLAMP TECHNIQUE IN FROG HEART. G. Salama* and M. Morad. Department of Physiology, Univ. of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19174.

Spacial uniformity of voltage control depends in part on homogeneity of voltage distribution in the tissue and separation of fluids at the sucrose-Ringer's interface. To evaluate the longitudinal distribution of potential, the electro-optical signal of merocyanine 540 was compared with the intracellular electrode response (Science 192:485, 1976). In the presence of TTX and 50/μM Ca, current pulses were applied across the gap. The intracellular potential and fluorescence signal were linearly related. In 0.8 - 1.0 mm strips, the time course of fluorescence waveform was slower in comparison to the intracellular potential. In 0.5 mm strips, the two signals are identical. The diffusion of sucrose into the Ringer pool was measured by scanning the fluorescence signal along the length of the muscle when 5 - 10 mM fluorescein sodium (FS, MW375) plus sucrose were perfused in the gap. FS diffuses through the extracellular space of the muscle from the sucrose to Ringer pool, but does not bind to the tissue. The concentration of FS in the physiological node is determined by comparing the fluorescence signal to that emitted by the muscle bathed in 5 mM FS. Fluorescence signal is linear with FS concentration up to 20 mM. The FS concentration within the first 25/μm from the sucrose-Ringer diaphragm ranged from 4.5 - 12% for 0.5 - 1 mmϕ strips. The diffusion profile is nearly exponential with the distance $\lambda = 190 \pm 25/\mu$m (in 4 muscles). Increasing the hydrostatic pressure across the gap by 3 mm H_2O does not alter the diffusion profile, but the maximum FS concentration at the interface changes by 2-3%. The results show that for small strips, the sucrose contamination across the diaphragm is small. The electro-optical method is useful in evaluating the voltage clamp technique.

W-AM-A8 NILE BLUE FLUORESCENCE SIGNALS IN FROG SINGLE MUSCLE FIBERS UNDER VOLTAGE OR CURRENT CLAMP CONDITIONS. J. Vergara* and F. Bezanilla, Laboratorio de Fisiología Celular, Universidad de Chile. Casilla 657, Viña del Mar, Chile.

The vaseline gap method described by Hille and Campbell (J.G.P., 67, 1976,265-293) was modified to record optical signals from single muscle fibers under voltage or current clamp conditions. Fiber were mounted across the three partitions of the lucite chamber and were illuminated by a narrow beam of quasi-monochromatic light in the region of pool A (Fig. 1A, Hille and Campbell, 1976). Fluorescence emission at 180° was collected by a 150 µm in diameter optical fiber in pool A. The signal to noise ratio in our records is large enough to allow characterization of the fluorescence signals produced by single stimulus pulses. Signals recorded when the muscle fibers were stimulated by brief current pulses started early in the falling phase of the action potential and lasted for up to 150 msec at 16° C. Under voltage clamp conditions, hyperpolarizing pulses failed to elicit detectable changes in fluorescence intensity. In Tetrodotoxin poisoned fibers the kinetics and magnitude of the fluorescence signal elicited by depolarizing pulses depended on the magnitude of the depolarization.Changes in Nile Blue fluorescence detected in single fibers are identical to the optical signals recorded in whole muscles (Bezanilla and Horowicz, J.P., 1975) The voltage dependence of the fluorescence signal is consistent with the hypothesis that Nile Blue A is monitoring the depolarization of the Sarcoplasmic reticulum's membranes.

(Supported by grants from Muscular Distrophy Associations of America, Inc. and Oficina Técnica de Desarrollo, Universidad de Chile)

W-AM-A9 CONTRACTILE ACTIVATION BY VOLTAGE CLAMP DEPOLARIZATION OF CUT MUSCLE FIBERS. L. Kovács* and M.F. Schneider, Dept. of Physiology, Univ. Rochester School of Medicine and Dentistry, Rochester, N.Y. 14642

The procedure for preparing cut frog skeletal muscle fiber segments introduced by Hille and Campbell (J. Gen. Physiol. 67: 265, 1976) has been modified. Fibers were cut after contracture and relaxation in a high K, Ca-free solution and mounted in a chamber so that about 500 µm of intact fiber, closed at the tendon, extended beyond a single vaseline gap (∿500-600 µm). A notch was cut in the fiber just beyond the other side of the gap. The cut end solution was (mM): 120 K glutamate, 2 $MgCl_2$, 0.5 ATP, 1 EGTA and 5 tris-maleate buffer (pH 7.0). Input voltage and current to the closed segment was determined from measured pool voltage and total current using a compensation circuit. Compensation was set with a microelectrode in the fiber. The electrode was then removed to permit voltage-clamping and electrical recording during fiber shortening without damage. In Ringer's + TTX at 3-4°C strength-duration curves for contraction threshold were similar to those reported by Costantin (J. Gen. Physiol. 63: 657, 1974) for intact fibers. Delayed rectifier currents were also observed. Using $I_K = n^4 \bar{g}_K (V-V_K)$ with $V_K = -80$ mV the n_∞ vs. V curve was similar to that determined in intact fibers (Chandler et al., J. Physiol. 254: 285, 1976). (Supported by N.I.H. and Muscular Dystrophy Association).

W-AM-A10 RELAXATION IN VOLTAGE CLAMPED MUSCLE FIBERS. Carlo Caputo. Centro de Biofísica y Bioquímica, Instituto Venezolano de Investigaciones Científicas (IVIC), Apartado 1827, Caracas 101, Venezuela.

Short fibers (1.5-2 mm) dissected from the lumbricalis muscle of the frog can be voltage clamped with two microelectrode technique (Bezanilla et al. Acta Cient. Venez. 22:72, 1971). The fibers were bathed in Ringer's solution containing TTX and held at a resting potential of -100 mV. Under these conditions, the effect of membrane potential on the rate of relaxation following tension induced by relatively short pulses was studied. The rates of relaxation after test pulses of amplitude sufficient to elicit maximal tension (60 to 100 mV) and duration between 100 and 600 msec were similar. With test pulses of 200 msec duration, the rate of relaxation was between 10 sec^{-1} and 20 sec^{-1}. In the case of two fibers in which maximal tensions were obtained at membrane potential values of -40 and -30 mV, the relaxation rates were 12.0 and 14.9 sec^{-1} respectively. In the first case, when the membrane potential during a second pulse applied immediately after the first one was -60 or -55, the relaxation rates were 11.9 and 5.2 sec^{-1} respectively. In the second case, the second pulse brought the membrane to -60 or -50 and the relaxation rates were 15.2 and 9.3 sec^{-1} respectively. In both cases, when the membrane potential during the second pulse was more negative than the holding potential, the relaxation rates were not affected. When the second pulse was less negative than the test pulse by less than 10 mV, the contraction was sustained and relaxation occurred only when the second pulse ended. These results indicate that relaxation rates are greatly decreased in the region of the membrane potential near the contractile threshold. This can be explained assuming that the decrease in the rates of relaxation are caused by liberation of extra amounts of contractile activator. (Supported by CONICIT grants N°31.26.S1-0296 and N°31.26.S1-0603).

W-AM-A11 EXTERNAL CALCIUM ION AND MECHANICAL ACTIVATION IN FROG SLOW FIBERS. W.F. Gilly* and C.S. Hui* (Intr. by S.M. Baylor), Dept. Physiology, Yale Univ. Medical School, New Haven, CT.

Effects of extracellular Ca^{++} on mechanical activation in slow muscle fibers of Rana temporaria were studied with a 2-microelectrode voltage clamp method. Threshold sarcomere shortening in response to membrane potential steps of various durations (2-800 msec) was determined by microscopic observation in slow (pyriformis) and TTX-poisoned twitch (sartorius) fibers at 20° and 9°C. Strength-Duration curves for the two fiber types are very similar in general and identical for brief pulses (<10 msec). Both slow and twitch fibers bathed in a Ca-free Ringer solution containing 2.5mM EGTA and 10.8mM $MgCl_2$ show a uniform 5 mv depolarizing shift of the S-D curve at all pulse durations. This shift is the same as that produced by adding 10mM $MgCl_2$ to normal 1.8mM $CaCl_2$ Ringer.

Short slow fibers from the cruralis muscle were also studied under voltage clamp, and tension was measured in response to membrane depolarization. Fibers show little relaxation during long (100-200 sec) depolarizations to 0 mv or beyond producing maximal tension. Removal of extracellular Ca^{++} with the EGTA-Mg Ringer has no effect on these "tonic" contractions. Thus, an influx of external Ca^{++} does not appear to be involved in the normal pathway of mechanical activation in frog slow fibers.

W-AM-A12 K^+ EFFLUX AND TENSION GENERATION IN VENTRICULAR MUSCLE. T. Klitzner* and M. Morad. Dept. of Physiology, Univ. of Pennsylvania School of Med., Philadelphia, Pa. 19174.

Ni^{2+} was chosen as a Ca^{2+} antagonist in frog ventricle because Ni^{2+} mimicked the effect of withdrawal of Ca^{2+} by decreasing tension, reducing the plateau potential, and prolonging the action potential (Fed. Proc. 35:613, 1976). Ni^{2+} does not interact with contractile proteins (J. Gen. Physiol. 50: 1709, 1967). Ni^{2+} (0.5 mM) appeared to enhance the Na^+ selectivity of the ventricular membrane as reflected by an increase in the overshoot (from 23±2 to 39±2 mV), an increase in the dependence of the overshoot on Na^+_o (from 22 to 57 mV/decade at 23°C) and the ability of TTX (10^{-6}M) to abolish both the upstroke and the plateau of the action potential. Studies using the single sucrose gap voltage clamp technique show that the voltage-tension (V-T) relation decreased by 30-40% in the presence of Ni^{2+}. Suppression of tension could be reversed by increasing Ca^{2+}_o. While TTX alone had little or no effect on tension, TTX in the presence of Ni^{2+} decreased the V-T relation to less than 15% of control. In presence of Ni^{2+} and TTX, increasing Ca^{2+} was relatively ineffective in restoring tension. Analysis of net membrane current shows that in the presence of Ni^{2+}, TTX decreases time dependent outward currents. The magnitude of the post-clamp after potential was measured to estimate what fraction of net membrane current is carried by K^+ (Science 191:190, 1976). The analysis showed that in the presence of Ni^{2+}, the TTX induced decrease in time dependent outward current may be quantitatively related to a decrease in K^+ efflux. The relation between the decrease in K^+ efflux and the decrease in tension caused by TTX in the presence of Ni^{2+} is linear. These results suggest that Ni^{2+} unmasks a TTX-sensitive Ca^{2+} transport mechanism which contributes to generation of tension. Ca^{++} influx through this transport system appears to be related to K^+ efflux. (GM02046 and HL 16152)

W-AM-A13 RAPID SODIUM-LOADING OF CARDIAC PURKINJE FIBERS AT 36°C AND THE RESULTANT STIMULATION OF ACTIVE SODIUM EXTRUSION. David Gadsby* and Paul F. Cranefield, The Rockefeller University, New York, N.Y. 10021.

The membrane responses of very thin bundles of canine cardiac Purkinje fibers to rapid changes in ionic environment were studied in a modified Hodgkin-Horowicz fast perfusion system. Cl-free solutions containing isethionate as the major anion were used to avoid the possible complication of chloride ion movements. After perfusion with K-free solution for longer than half a minute an extra hyperpolarization was recorded on returning to K-containing solution. This hyperpolarization reached a maximum value in a few seconds; the potential then more slowly declined to the control level. Both the rate of establishment and magnitude of the extra hyperpolarization seen on return to a given $[K]_o$ were larger after longer exposures to zero $[K]_o$. After a given duration of zero $[K]_o$ exposure the rate of rise of the extra hyperpolarization increased as the level of $[K]_o$ to which the fiber was returned was raised (up to 12mM) but its magnitude, although increasing with $[K]_o$ in the range 0-4mM, declined when $[K]_o$ was raised further. The extra hyperpolarization was reversibly abolished by 10^{-5}M acetyl strophanthidin. A return to 4mM $[K]_o$ following a 5min exposure to zero $[K]_o$ is accompanied, in driven preparations, by a shortening of the action potential (as much as 30% reduction in duration at 50 beats/min) which may persist for several minutes. All of these phenomena presumably result from the electrical effects associated with active sodium extrusion. (supported by HL-14899)

W-AM-A14 INTRACELLULAR $[Cl]_i$ IN MUSCLE: QUANTITATIVE ELECTRON PROBE ANALYSIS OF CRYO SECTIONS. Avril V. Somlyo* H. Shuman* and A.P. Somlyo, Pennsylvania Muscle Institute, Presbyterian-University of Pennsylvania Medical Center, 51 North 39th Street, Philadelphia, Pa. 19104

Quantitative electron probe analysis of unfixed cryo sections was used to determine whether cellular Cl content was in agreement with a passive distribution and whether compartmentalization in organelles could account for the "excess Cl" measured with previous bulk chemical analyses and flux studies. The $[Cl]_i$ (determined with 0.5-9μm diameter electron probes) of frog EDL and semitendinosus (in frog Ringer's, K_o=3mM) was 24 ± 1.1S.E.mmoles/Kg dry wt. (n=34). The $[Cl]_i$ determined with small (50-100nm) diameter probes over cytoplasm excluding organelles or over nuclei or terminal cisternae (T.C.) were not significantly different. Mitochondria partially excluded Cl, with cytoplasmic/mitochondrial Cl ratio of 2.4±0.8S.D.Calcium (66mmoles/Kg dry wt. ± 4.6S.E.) was detected in T.C. Hypertonic 2.2x NaCl, 2.5x sucrose or 2.2x Na isethionate produced: 1)swollen T.C. containing significantly higher than cytoplasmic concentrations of Na and Cl or S (isethionate); 2)absence of detectable Ca in the swollen T.C.; 3)granules of Ca, Mg and P≃(6Ca+1Mg)/6P in the longitudinal SR.

In rabbit portal vein smooth muscle bathed in 5.9mM K_o Krebs, the $[Cl]_i$ was 180mmoles/Kg dry wt. ± 3.8S.E. (n=13); cytoplasmic K/Cl was 2.9. In Krebs + 80mM KCl the fibers (n=5) gained 250 K and 90 Cl (mmoles/Kg dry wt.) approaching a Boyle-Conway distribution. In conclusion: 1)the cytoplasmic [Cl] of smooth and striated muscles in low $[K]_o$ solutions exceeds that predicted by a passive electrochemical distribution; 2)there is no evidence of sequestered Cl in the T.C. of normal muscles but Cl is sequestered in the swollen T.C. of hypertonically treated muscles; 3)hypertonicity translocates Ca from the T.C. into the longitudinal tubules.

(Supported by HL-15835 to the Pennsylvania Muscle Institute)

W-AM-A15 EVIDENCE FOR THE EXTRACELLULAR COMPOSITION OF THE FLUID IN THE SARCOPLASMIC RETICULUM OF FROG SARTORIUS MUSCLE. Margaret C. Neville. Department of Physiology, University of Colorado Medical Center, Denver, Colorado 80262.

Compartmental analysis of curves for the efflux of Na, Cl, sucrose, 3-O-methylglucose, xylose, cycloleucine, leucine, and glycine from frog sartorius muscle reveals three components. The slowly effluxing component can be identified with solute leaving the intracellular compartment. The rapidly effluxing component is consistent with solute efflux from the extracellular space. The intermediate component has the following characteristics: 1) The efflux rate is about 6 times slower than efflux from the extracellular space for all solutes examined. 2) The distribution space was similar for all solutes examined -- about 8.7% of the muscle volume in summer frogs. This value is consistent with stereological estimates of the sarcoplasmic reticulum volume by Mobley and Eisenberg (J. Gen. Physiol. 66, 31-45, 1975). 3) The solute distribution space is increased in winter frogs and by hypertonic solutions. 4) The efflux rate is proportional to the diffusion coefficient of the solute in free solution. 5) Both the sucrose distribution space and its rate of efflux are decreased by about one-half in muscles pretreated with 400 mM glycerol. These data are consistent with the hypothesis that the composition of the fluid in the sarcoplasmic reticulum is similar to that of the extracellular medium and is maintained that way by non-specific channels between the sarcoplasmic reticulum and the t-tubular system. Supported by NIH grant AM 15907 and RCDA 5K04 AM 00038.

W-AM-A16 SYMPATHOMIMETIC RECEPTORS ON SKELETAL FROG MUSCLE FIBERS. R. Valle-Aguilera* and H. González-Serratos, Dept. of Pharmacol. and Dept. of Physiol., Centro de Investigación y de Estudios Avanzados del IPN, México D.F. México.

Isolated twitch muscle fibers of the frog show an increase in twitch tension when exposed to adrenaline ($1X10^{-5}M$). The increment in twitch tension could be attributed to a direct effect of cyclic AMP either on the contractile proteins or on the SR. To further characterize this effect, norepinephrine (α receptor stimulator), isoproterenol (β receptor stimulator) and epinephrine (α and β receptor stimulator) were used. The β and α + β receptor stimulators produced an increment on twitch tension (T), maximum rate of tension development ($+\dot{V}$) and maximum rate of tension decay ($-\dot{V}$). The results expresed as fractions of the controls are sumarized in the following table.

		T	$+\dot{V}$	$-\dot{V}$
norepinephrine	(α)	0.067	-0.047	-0.080
isoproterenol	(β)	0.16	0.208	0.180
epinephrine	(α+β)	0.166	0.198	0.322

It is clear that these muscle fibers have mainly β adrenergic receptors. The increase of maximal rates of tension development and decay are consistent with the idea that these drugs may act by pumping more Ca^{++} into the SR. In experiments with split muscle fibers, cyclic AMP produced an increment of tension development when Ca^{++} was subsequently released from the SR by caffeine. This experiment further supports the above sugestion.

W-AM-A17 COMPARISON OF THE INTERACTION OF NATIVE AND L_2-DEFICIENT MYOSIN WITH REGULATED ACTIN. Suzanne M. Pemrick, Departments of Biochemistry and Medicine, Mount Sinai School of Medicine of the City University of New York, New York, New York 10029.

Skeletal actomyosin prepared from L_2-deficient myosin and regulated actin requires a 5-fold greater free Ca^{++} concentration to attain half-maximal activation and maximal activation of the ATPase activity is reduced compared to acto-"native" myosin. L_2-deficient myosin (1 mole L_2/mole) can be prepared by DTNB-treatment without impairing the hydrolytic site although actin interaction in the presence of 10 μM free Ca^{++} is decreased from 0.05 to 1.0 mM MgATP. Neither the MgATP concentration at which calcium-sensitivity becomes apparent, nor the ATPase at millimolar MgATP in the absence of calcium is affected. This suggests the altered complex is an M.ADP.P intermediate. Equivalent results were obtained for many preparations of L_2-deficient myosin and regulated actin.

The hydrolytic activities (K-Ca-Mg-ATPases) of all "native" myosin preparations were similar. In the presence of regulated actin variations were observed in the absence of Ca^{++} with respect to ATP-inhibition of actin interaction. From 50 to 100 μM MgATP, some "native" myosin preparations exhibited an extended plateau for the ATPase activity. Since in the absence of actin, myosin was saturated with ATP, actin-interaction should have been minimal. One interpretation is that a small percentage of "native"-myosin molecules were "ATP-resistant". This species was detectable only in an ATP-dependency study. Neither calcium-sensitivity, nor the per cent relaxation were affected appreciably. Therefore, it is suggested that the L_2 light chain stabilizes an M.ADP.P intermediate and thereby modulates thin filament regulation from 0.01 to 1 μM free Ca^{++}.

(SMP is a Fellow of the New York Heart Association).

W-AM-B1 STRENGTH-FREQUENCY RELATIONSHIP FOR WHITE NOISE STIMULATION OF SQUID AXONS.
Rita Guttman, Ronald Grisell* and Lance Feldman*, Department of Biology, Brooklyn College of the City University of New York, Brooklyn, N.Y. 11210; Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550 and Marine Biological Laboratory, Woods Hole, Mass. 02543

The strength-frequency (input-output) relationship for a patch of space-clamped squid axon membrane, stimulated by continuous white noise current with Gaussian amplitude distribution, both by itself and superimposed on a step, was investigated experimentally and compared with 1) computations based on the Hodgkin-Huxley formulation and 2) the theoretical study of Stein, who used finite duration quantal inputs whose turn-on times were Poisson distributed. All three give similar sigmoid curves with ultimate saturation, although the theoretical curves lie to the left of the experimental, indicating greater sensitivity of the models to this form of input. Pre- and post-spike trajectories were compared at various noise stimulus intensities. The experimental results may be pertinent to models for sensory or synaptic behavior.
Aided by NIH grant 5R01NS12272.

W-AM-B2 SODIUM TAIL CURRENTS AND GATING CURRENT 'OFF' RESPONSES IN MYXICOLA GIANT AXONS.
C. L. Schauf, J. O. Bullock, and T. L. Pencek. Departments of Physiology and Neurological Sciences, Rush Medical College, Chicago, Illinois, 60612.

Sodium currents following repolarization to more negative potentials after initial activation of g_{Na} (Na^+ tails) were digitally recorded in voltage-clamped Myxicola giant axons compensated for series resistance. The results are inconsistent with a Hodgkin-Huxley type kinetic scheme. At potentials more negative than -50 mv the Na^+ tails (after subtraction of non-Na^+ currents) show two distinct time constants, while at potentials more positive than -50 mv only a single exponential process can be resolved. In a given axon the time course of the sodium tail currents were totally unchanged when g_{Na} was reduced by low [TTX] to as little as 10% of its initial value, demonstrating adequacy of series resistance compensation and lack of other current-dependent artifacts. Tail currents were altered by changes in $[Ca^{++}]$ in a manner consistent with a simple alteration in surface potential of a magnitude comparable to that derived from shifts in $g_{Na}(V)$. We could observe no dependence of the time course of the sodium tails on the time of repolarization relative to peak g_{Na} at any $[Ca^{++}]$. Repolarization becomes slower as the potential is made more positive over the range -160 mv to 0 mv, while the rate of sodium activation increases from -40 mv to 0mv. Asymmetry current 'OFF' responses measured in intact axons exposed to 10mM 4-aminopyridine to block I_K are well described by a single exponential with no sign of a slow component similar to that seen in the Na^+ tails. The time constant for the 'OFF' response averaged 2.3 times greater than the time constant for the rapid component of the Na^+ tails, with no evidence of a dependence on pulse amplitude or duration. The average value of τ_{off} at -100 mv (5°C) was 122 ± 3 μsec. Maximum charge movement was 0.34 ± 0.03 ncoul/mmho g_{Na} with the Q(V) having a slope of 22-26 mv/e-fold in axons where the $g_{Na}(V)$ slope was 9-11 mv/e-fold.

W-AM-B3 EFFECTS OF INITIAL CONDITIONS ON THE SODIUM CONDUCTANCE KINETICS IN MYXICOLA.
L. Goldman, and R. Hahin*, Dept. of Physiology, School of Medicine, Univ. of Maryland, Baltimore, Maryland 21201.

Effects of de- and hyperpolarizing conditioning pulses on the g_{Na} time course during test steps were studied over a 150 mV range of conditioning potentials for conditioning durations, t_c, of 50 μsec to 30 msec. For most of the range studied conditioning polarization produced a simple translation (Δt) of the normalized $g_{Na}(t)$ along the time axis. For the g_{Na} rise, this was true throughout. Additionally, for large conditioning depolarizations the inactivation time constant during the test pulse decreased. There were no other effects. Hodgkin-Huxley kinetics (Myxicola parameters) predict simple time shifts but of a magnitude some 3-fold greater than found experimentally. Coupled kinetics (Goldman, Biophys. J. 15: 119, 1975) also predict a simple time shift, different from that found experimentally, but with better agreement than for m^3h kinetics, and do display a rough predictive value, suggesting that the time shift is a property of the gating machinery. Δt vs. t_c can be described as a single exponential. Semi-log plots of $\Delta t - \Delta t_\infty$ generally extrapolated to a residual Δt at $t_c = 0$, suggesting that there is an additional process too rapid to resolve in these experiments. Time constants of the Δt dependency on t_c were 3-5 times faster than predicted by m^3h kinetics in the depolarizing range and also 1.5-2 times faster than predicted by coupled kinetics. These results and other discrepancies between experiment and predictions from coupled kinetics at short conditioning polarizations are consistent with the idea of an additional state in the gating unit intermediate between rest and conducting, which is itself non conducting. This view is also consistent with recent results on the gating current. However, other interpretations of these data are also possible, and this idea may be taken as tentative only. (Supported by USPHS Grant NS-07734).

W-AM-B4 NA-CURRENT FLUCTUATIONS GIVE ESTIMATES OF SINGLE-CHANNEL CONDUCTANCE IN FROG NERVE F.J. Sigworth*(Intr. by C.F. Stevens), Department of Physiology, Yale School of Medicine, New Haven, Connecticut 06510

The inactivation of Na currents prevents the use of conventional noise analysis techniques to determine the single-channel conductance (γ) of unmodified Na channels at large depolarizations. However, the variance of an ensemble of Na current records can be used to estimate γ. Frog nodes were voltage clamped at 2°C in the presence of TEA with repetitive depolarizing pulses; the resulting current records were filtered at either 2 or 5 KHz and digitized. The mean and variance of the measured currents at each time point were calculated, and the membrane conductance-dependent contribution of thermal noise to the variance was subtracted. If it is assumed that Na channels have only two conductance states and are independent, and that the measured fluctuations are due only to their discrete conductance changes, values of single-channel conductance in the range of 4-10 pmho are obtained for depolarizations between 20 and 75 mV. The value of γ is constant as inactivation develops, and is not significantly changed by prepulses in the range of -35 to +15 mV referred to resting potential.

W-AM-B5 IDENTIFICATION OF SODIUM ION CONDUCTION NOISE IN THE SQUID AXON. L.E. Moore, H.M. Fishman, and D. Poussart,* Dept. of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas, and Laval University, Dept. of Electrical Engineering, Quebec, Canada.

Under normal ionic conditions in the squid axon, sodium conduction fluctuations are masked by significantly larger K^+ conduction fluctuations, which consist of both relaxation and f^{-1} components. It was found that the most effective condition for observing sodium conductance fluctuations is one in which all potassium related conductance fluctuations are suppressed. This cannot be achieved with a blocker of the potassium current such as tetraethylammonium ion (TEA) which produces excess noise in the presence of internal K^+ by virtue of its interaction with the conducting channel. An effective method to suppress the K^+ and leakage systems and enhance the observation of Na^+ current noise was found to be an internal perfusion of either CsF or tetramethylammonium fluoride (TMA) completely replacing the internal K^+ to prevent any induced fluctuation of the K^+ current by the above mechanism. Spectral analysis of the spontaneous current fluctuations from small, isolated patches of these axons at 5°C yields a TTX sensitive noise component in the 100-1000 Hz range. The intensity of the TTX sensitive noise component is related to the magnitude of the inward current, increasing to a maximum for a depolarization to about -20mV and decreasing on further depolarization or hyperpolarization. Difference spectra calculated by subtracting the residual spectrum at the holding potential (-100mV) from spectra measured at depolarized levels show apparent Lorentzian power spectra with estimated corner frequencies of 500-1000 Hz. Since the Na^+ current noise observed with either CsF or TMA was essentially the same, it is unlikely that this fluctuation component is an induced noise.

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W-AM-B6 MEASUREMENT OF THE COMPLEX ADMITTANCE OF EXCITABLE MEMBRANE - AN EFFICIENT METHOD. D. Poussart*, L.E. Moore, and H.M. Fishman, (Intr. by D. Baker), Université Laval, Quebec, Canada and University of Texas Medical Branch, Galveston, Texas 77550.

The small-signal admittance provides a complementary probe to the study of ionic and charge movements and their kinetics in excitable membrane. Previous measurements were restricted to near rest potential due to lengthy data acquisition time. An efficient method, with speed near the theoretical limit for time-frequency domain analysis, was developed and applied to squid axon under axial-wire, voltage-clamp conditions. A 1 mV p-p pseudo-random binary sequence (PRBS) is superposed as a small perturbation on a normal step clamp. The response is sampled and processed by digital Fourier transform while maintaining precise register between the PRBS and the sampling window through a form of time compression-expansion. Computations, which are made digitally in 90 msec, yield absolutely calibrated magnitude and phase data simultaneously over 256 discrete frequency components. Membrane admittance was measured from .1 to 2560 Hz, the upper frequency range requiring perturbations of only 100 msec. As a result of high speed, extensive data can be obtained for membrane potentials from -100 mV to beyond +30 mV. In external SW, the interaction of K and Na conductances with membrane capacity reflects in a pronounced anti-resonance. This feature is modified considerably but not eliminated by external application of TTX (1 μM). Internal perfusion with .29 M CsF produces conduction which is dominated by the Na process with a low frequency phase which increases beyond 180° in correlation with a steady-state inward current condition. Such data provide an alternative, detailed (linearized) description of the various conduction processes.

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W-AM-B7 CHARGE MOVEMENTS AND ADMITTANCE IN SQUID AXON. H.M. Fishman, L.E. Moore, and D. Poussart,* Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550 and Dépt. de Génie Électrique, Université Laval, Québec, Canada.

The complex admittance $Y(j\omega)$ of squid axon was measured by guarded axial-wire technique and application of small-signal broadband noise during voltage clamp (see Poussart et al., these abstracts). Measurements were made at 10°C before and after ion conduction suppression by internal perfusion with Cs solutions and with Na and K-free ASW containing TTX (1μM) externally. Admittance measurements were made only when the current response to paired step clamp pulses of ± 70mV from a holding potential of -100mV produced a difference current (i.e., "gating" current). The ratio of charge moved (obtained from the difference current) to potential change yields an apparent capacitance of .36μF/cm^2. In squid axon (Keynes, Rojas & Rudy, J. Physiol. 239: 100p, 1974) and in lipid bilayers (Szabo, these abstracts), as holding potential (-100mV) is depolarized toward 0mV, immobilization of membrane charge by the field is reduced, and capacitance, measured in the steady state, is expected to increase. However, admittance measured at 60mV or more depolarized from holding potential (-100mV) in the frequency range 100-2500 Hz indicates a decrease in capacitance of .07 to .15μF/cm^2 from that obtained at holding potential. Within the context of present views of "gating" current, the admittance measurements are reconciled if some type of "inactivation" process occurs (Bezanilla & Armstrong, Phil. Trans. R. Soc. Lond. B. 270: 449, 1975) in which the membrane charge, available for movement, decreases at depolarized holding potentials.

Aided by NS-11764, NS-13520, and CNRC grant A5274.

W-AM-B8 ADMITTANCE AND STEP-CLAMP RESPONSE FOR ION REDISTRIBUTION IN BILAYERS: A SIMPLE MODEL FOR GATING CURRENTS. Gabor Szabo, Dept. of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

Admittance and small step response due to ionic current induced by the hydrophobic anion tetraphenylborate in cholesterol-monoolein bilayers, were characterized at various levels of a steady holding potential. Both techniques yield identical results in that the effect of TFB can be represented by a voltage-dependent series RC branch in parallel with the intrinsic bilayer capacitance. R is minimal (~2 kΩcm^2) while C is maximal (~0.4 μF/cm^2) for holding potentials that result in zero field across the membrane. These results agree with expectations for a first order translocation process across the bilayer of TFB trapped at the membrane surfaces (Ketterer et al., J. Memb. Biol. 5: 225, 1972). The bilayer models the gating currents of the squid axon since both the magnitude and the time course of difference current, obtained by the procedures used to measure gating currents, are similar to those observed for squid axon. Furthermore, the difference current vanishes and changes polarity as the holding potential is incremented through the value for which the field is zero in the membrane, in a fashion similar to that observed by Keynes et al. (J. Physiol., 239: 100p, 1974). However, although difference currents vanish, individual step clamp responses are maximal (but equal in opposite directions) for this holding potential. Difference currents must thus be interpreted with care since these do not always represent the total charge transferred during a single pulse. The bilayer model predicts a measurably increased capacitance near zero-field holding potentials. The fact that this is not observed in the squid axon (Fishman et al., these abstracts) may result from inactivation of charge movement in the axonal membrane.

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W-AM-B9 A QUANTITATIVE MODEL-INDEPENDENT REPRESENTATION OF SODIUM CONDUCTANCE AND INACTIVATION KINETICS. E.G. Jakobsson, Department of Physiology and Biophysics and Bioengineering Program, University of Illinois, Urbana, Illinois 61801.

By current response to voltage clamp, two properties of the rapid transient sodium conductance system may be determined. They are: i) conductance and ii) potential for conductance increase (i.e., inactivation level). It is thus suggested that a sodium "channel" may exist in one of at least three states: α) not conducting but potentially activatable by a depolarization, β) conducting, or γ) neither conducting nor activatable (i.e., inactivated). In Hodgkin-Huxley parameters it is reasonable to postulate that the fractional population of the sodium system in each of the α, β, and γ states above is h - $g_{Na}/\bar{g}_{Na}$, $g_{Na}/\bar{g}_{Na}$, and 1 - h, respectively. Voltage clamp data from a "typical" Myxicola giant axon (kindly provided by Dr. C. L. Schauf) are represented as the time course of these three populations and it is seen that the time course of the "α" state is triphasic, suggesting at least four possible states of the system. Kinetic and energetic arguments suggest that the fourth state comes from a splitting of the "α" state into a high and low energy component, and that the high energy component has a very low steady state population but a very high population immediately following a depolarizing voltage step. This high energy component is thus an "excited" rather than an equilibrium state, and is the immediate precursor of the conducting state. The notion that the immediate precursor of the conducting state is an excited state is further supported by calculating the rate of free energy change after a voltage step, i.e., $\dot{F} = RT\,(\ln((\beta\alpha_\infty)/(\alpha\beta_\infty))\,\dot{\beta} + \ln((\gamma\alpha_\infty)/(\alpha\gamma_\infty))\,\dot{\gamma})$. During the rising phase of g_{Na}, F is positive, suggesting that the reaction during that time is something other than a relaxation of equilibrium states. Partially supported by Research Board of the University of Illinois.

W-AM-B10 *IN VITRO* DEVELOPMENT OF FAST NA^+ CONDUCTANCE IN EMBRYONIC HEART CELL AGGREGATES. Richard D. Nathan and Robert L. DeHaan, Dept. of Physiology, Texas Tech Univ. School of Med. Lubbock, TX 79409 and Dept. of Anatomy, Emory Univ. School of Med., Atlanta, GA 30322.

In previous studies it has been reported that the electrophysiological properties of young embryonic heart cells maintained as monolayers, reaggregates, or in organ culture, fail to mature normally *in vitro*[1]. We have applied a double-microelectrode voltage clamp technique to spheroidal aggregates of 3-day chick heart cells after two days of gyration (3+2 day) and to similar aggregates after an additional three days of gyration (3+5 day), and have shown that a fast sodium conductance does develop *in vitro*. In four experiments, only a single slowly-activated and slowly-inactivated inward current was recorded from 3+2 day aggregates. This inward current was maximal between -40 and -30 mV and decayed with a time constant of about 12 ms at -35 mV. By contrast 3+5 day aggregates always exhibited two components of inward current, one of which was rapidly activated and inactivated (τ = 1.6 ms at -30mV) and one which was slowly activated and inactivated (τ = 25.4 ms at -30mV). The fast inward current was maximal near -30 mV and the slow inward current near 0 mV. The kinetics and voltage dependence of these two currents were similar to those recorded from control aggregates prepared from 7-day embryonic hearts. Additional experiments showed that the inward current recorded from 3+2 day aggregates was not blocked by TTX (10^{-5}g/ml) while the fast inward current in 3+5 day aggregates was. In 34 intracellular recordings from 3+5 day aggregates the maximum upstroke velocity was 110.3 ± 3.5 V/s (mean ± SE), which was comparable to values recorded in 7-day controls. (Supported in part by NIH Postdoctoral Fellowship HL-01321 to R.D.N. and by grants HL-16567 and HL-17827 to R.L.D.)

[1]M. J. McLean, J. F. Renaud, N. Sperelakis, and M. C. Niu, Science 191: 297 (1976).

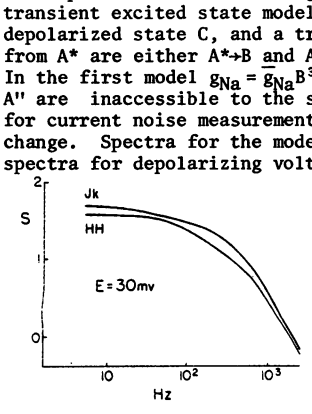
W-AM-B11 LITTLE SODIUM STORAGE OCCURS WITHIN THE AXONAL MEMBRANE. Franklin F. Offner, Technological Institute, Northwestern University, Evanston, Il. 60201.

Evidence indicates that Na+ activation is at the external interface of the axon membrane (TTX), and inactivation at the internal (pronase[1]). The Na+ storage hypothesis is consistent with this, and tracer experiments have been used to support the hypothesis,[2] which if proved would have implications as to channel selectivity.[3] Since the hypothesis implies that Na+ flow is responsible for inactivation, little inactivation should occur when depolarizing to a high positive internal potential; the Hodgkin-Huxley equations predict rapid inactivation. The extent of inactivation can be determined by subsequent repolarization to an intermediate potential, e.g. 0 mV; the storage model predicts Na+ inflow, while H-H predict none. This experiment has been performed[4] on *myxicola* axon, with external ASW and with ASW + TTX. The results indicate that at most only a small portion of the ion flow is stored, and that the time constant of inactivation cannot be due to storage time, but probably to a time of response of the inactivation gates. This would bring the predicted channel density more nearly into agreement with calculations of others.

1. Armstrong, Bezanilla and Rojas, J. Gen. Physiol. 62:375 (1973).
2. Cohen and Landowne, J. Physiol. 236:95(1974).
3. Offner, J. Physiol. 252:69P (1975).
4. Results kindly provided by Dr. Charles Schauf.

W-AM-B12 A COMPARISON OF ION CURRENT NOISE PREDICTED FROM DIFFERENT KINETIC MODELS OF THE SODIUM CHANNEL. J.R. Clay, Anatomy Dept., Emory Univ., Atlanta, GA 30322.

A noise analysis of fully coupled transient excited state models of the Na channel (Jakobsson, Biophys.J.16:78a,1976); _____, in press) will be given and compared with Na ion noise predicted from the Hodgkin-Huxley equations (Hill & Chen, Biophys.J.12:948,1972). The transient excited state models consist of a hyperpolarized state A, a conducting state B, a depolarized state C, and a transient state A* which is coupled to A by A↔A*. The transitions from A* are either A*↔B and A*↔C, or A*↔A'↔A''↔B and A*↔C in one variation of the basic model. In the first model $g_{Na} = \bar{g}_{Na} B^3(t)$ and $g_{Na} = \bar{g}_{Na} B(t)$ in the latter model. The states A*, A' and A'' are inaccessible to the system under the steady state voltage clamp conditions required for current noise measurements because A↔A* depends directly upon rate of membrane voltage change. Spectra for the model with $g_{Na} \simeq B^3(t)$ ($B_\infty = m_\infty h^{1/3}_\infty$) are very similar to H-H Na spectra for depolarizing voltages E of 0 to 15 mV. Differences are apparent for 20 mV < E < 35 mV, as shown in the insert (S = log S(f)), which may, however, be of the same order of magnitude as errors involved with present-day experimental techniques. Spectra for $g_{Na} \simeq B(t)$ ($B_\infty = m^3_\infty h_\infty$) are very different than HH with roll-off frequencies of ≃300 Hz at 6.3°C. If the two transient state models are shown to have similar macroscopic behavior, current noise may be a sensitive test of their different microscopic behavior.



Supported by NIH (HL05346-01A1)

W-AM-B13 A COMPARISON OF THE pH DEPENDENCE FOR BLOCK OF SODIUM CHANNELS IN LOBSTER AXONS BY H^+ ION AND DYE-SENSITIZED PHOTOOXIDATION. J. P. Pooler and D. P. Valenzeno, Physiology Department, Emory University, Atlanta, Ga. 30322.

Sodium channels in nerve membranes can be blocked reversibly by H^+ ions and irreversibly by dye-sensitized photooxidation, the latter with a rate which is a function of pH. The pH dependence for both kinds of block was studied on lobster axons using the double sucrose gap, voltage clamp technique at buffer concentrations of 20 mM. Low pH depressed sodium currents relative to those at pH 8 over the potential range -40 mv to +90 mv. Using the relative slope conductance at +20 mv as an assay the apparent pK for H^+ block is near 4.8. The block is voltage dependent, being relatively less at increasingly more positive potentials. Photooxidation was carried out using the sensitizer eosin Y at 5 μM concentration. Its magnitude was measured during several seconds of illumination as the rate constant for decline in values of peak sodium current flowing through non-H^+ blocked channels, assessed by repetitive depolarizations to a potential on the positive limb of the I-V curve. At low pH the rates of photooxidation were reduced relative to those at pH 8, with a pK similar to that for H^+ ion block. At high pH the rates were also reduced, with a pK near 10.4. High pH alone had no obvious effect. These results indicate that the photooxidizability of sodium channels depends on the ionization status of two separate H^+ binding sites, one of which could be identical to the site for block by H^+.

W-AM-B14 ASYMMETRIC IONIC CONCENTRATION DEPENDENCE OF SODIUM CHANNEL SELECTIVITY. M. Cahalan and T. Begenisich, Dept. of Physiol., Univ. of Penna., and Dept. of Physiol., Univ. of Rochester.

We have continued our efforts to determine the dependence of sodium channel selectivity on changes in concentration of both internal and external ions. The experiments were performed on voltage-clamped internally perfused squid giant axons. The ionic permeability ratios (e.g. P_{Na}/P_{NH_4}) were calculated from the sodium channel zero current (reversal) potential using the Goldman, Hodgkin, Katz equation. Changes in intracellular $[Na^+]$ (with fixed external $[NH_4^+]$ and changes in external $[NH_4^+]$ (with fixed internal [Na]) do not alter the P_{NH_4}/P_{Na} ratio (P_{NH_4}/P_{Na} is about 0.8). However, alterations in internal $[NH_4]$ (with constant external [Na]) modify the P_{NH_4}/P_{Na} ratio. Also, under certain conditions (e.g. with 275 mM Rb_i, 10 NH_{4_i}, or 48 K_i) external Na can affect the P ion/P_{Na} ratio, possibly due to a membrane potential effect on sodium channel selectivity. Preliminary theoretical calculations have shown that these results do not necessarily require a structural change of the Na channel selectivity filter, but could arise from non-independent ionic movements. Supported by NIH grants NS-12547-01 and 5P01NS10981.

W-AM-B15 GATING CURRENTS: THE ROLE OF NONLINEAR CAPACITATIVE CURRENTS OF ELECTROSTRICTIVE ORIGIN. F.J. Blatt, Physics Dept. and Neuroscience Faculty, Michigan State University, East Lansing, MI 48824

The non-linear capacitative currents deriving from electrostrictive changes of membrane capacitance have been calculated under conditions similar to those employed by Armstrong and Bezanilla [1] and Keynes and Rojas [2]. Calculations were performed under two limiting conditions. In the first it is assumed that the membrane thickness responds instantaneously to changes in voltage across the idealized membrane capacitance. In the second it is assumed that the membrane thickness approaches a new equilibrium value consistent with membrane voltage exponentially with a time constant long compared to that of the charging circuit. For values of the parameter characterizing membrane electrostriction in the range suggested by optical retardation studies [3] the non-linear current of electrostrictive origin is comparable in magnitude and time course, but is opposite in direction to the observed gating currents. Hence, a priori neglect of electrostrictive currents may not be justified, but the presence of these currents would imply even larger gating currents than deduced earlier [1], [2]. Conversely, if electrostrictive currents are, in fact, negligibly small, the compressibility of biological membranes must be rather small, probably less than 4×10^{-6} m^2/N. In that case the optical retardation results of Cohen et al. [3] cannot be attributed to electrostriction.

[1] C.M. Armstrong and F. Bezanilla, J. Gen. Physiol. 63, 533 (1974).
[2] R.D. Keynes and E. Rojas, J. Physiol. (London), 239, 393 (1974).
[3] L.B. Cohen, B. Hille, R.D. Keynes, D. Landowne and E. Rojas, J. Physiol. (London) 218, 205 (1971).

W-AM-B16 CALCIUM AFFECTS KINETICS OF SODIUM ONSET GATES, BUT NOT RESET GATES.
J. W. Moore, M. Westerfield, S. Jaslove, Department of Physiology, Duke University, Durham, N. C. 27710

Intact squid axons under voltage control in a sucrose gap produce larger (than those reported for perfused axons) asymmetrical displacement or "gating" currents. Electronic subtraction of leakage and multiple time constant capacitative currents allows "onset gates" to be seen preceding normal sodium currents. TTX and 4AP are added to the external perfusate for kinetic studies of the "gates". A computer runs the experiments, averages to improve signal-to-noise, and subtracts residual bridge unbalance.

Changing the $[Ca^{++}]_o$ level affects the kinetics of both on and off sodium conductances and may help to discern the sodium "gating" component of the asymmetry current. We found that changing from 10 to 150 mM $[Ca^{++}]_o$:

1) reduces the peak slightly and slows the decay of the onset "gating current"

2) does not change the time constant of the "tail gates"

3) causes the time constant of the sodium tails to equal that of the "tail gates" for some, but not all, repolarizing voltages.

W-AM-C1 LIMULUS PHOTORESPONSES OBTAINED AFTER INTRACELLULAR INJECTION OF CA^{++} MIMIC THOSE OBTAINED AFTER LIGHT ADAPTATION. J. Sherwood Charlton* and Alan Fein, Laboratory of Sensory Physiology, Marine Biological Laboratory, Woods Hole, Mass. 02543.

Calcium ions were iontophoretically injected into ventral photoreceptors of Limulus by passing current between two intracellular pipettes. The receptor responses (late receptor potential) to brief flashes of light were obtained during interruptions of the calcium ion injections and during light adaptation and compared to control responses. Control responses were obtained after recovery from the effects of the Ca++ injection or the light adaptation. Changes in receptor sensitivity and in the photoresponse time course produced by Ca++ injections were compared to changes produced by light adaptation. It was found that for equal decreases in sensitivity produced by light adaptation or Ca++ injection, the photoresponses to the same stimuli had similar response waveforms. The response latencies obtained during both light adaptation and Ca++ injections were found to be shorter than the latencies of the controls. The variability of the responses obtained during both light adaptation and Ca++ injections were found to be less than the variability in the control responses. For an equal desensitization, the photoreceptor has a similar light intensity-response characteristic whether desensitized by injected calcium ions or light adaptation. Under the conditions of these experiments, the intracellularly injected calcium ions approximately mimic light adaptation.

W-AM-C2 THE ROLE OF INTRACELLULAR NA^+ IN LIGHT AND DARK ADAPTATION IN LIMULUS VENTRAL PHOTORECEPTORS. Alan Fein and J. Sherwood Charlton*, Laboratory of Sensory Physiology, Marine Biological Laboratory, Woods Hole, Mass. 02543.

Limulus ventral photoreceptors were intracellularly iontophoretically injected with Na^+, by passing current between two intracellular pipettes. The photoresponse (light induced inward membrane current) of the receptor was measured under voltage clamp. The changes in receptor sensitivity and photoresponse time course produced by Na^+ injection, were compared with light, dark and local adaptation produced by illumination. Our results indicate that Na^+ injection produces changes in sensitivity and time scale similar to light adaptation. The time course of recovery of sensitivity from Na^+ injection was found to be much slower than a comparable dark adaptation. Also, unlike light and Ca^{++}, Na^+ injection did not produce localized desensitization of the photoreceptor. These results and others suggest that changes in intracellular Na^+ concentration probably contribute less than 10% to light and dark adaptation in this photoreceptor.

W-AM-C3 ALTERATION OF SENSITIVITY AND TIME SCALE IN INVERTEBRATE PHOTORECEPTORS EXPOSED TO ANOXIA, DINITROPHENOL AND CARBON DIOXIDE. R. Clark Lantz* and Alexander Mauro, The Rockefeller University, New York, N.Y., 10021

The effects of anoxia, the metabolic inhibitor, 2,4-dinitrophenol (DNP), and of carbon dioxide (CO_2) on the late receptor potential of several invertebrate photoreceptors have been studied. These studies were carried out by recording intracellularly from photoreceptors in the lateral ocellus of Balanus, and in the lateral and ventral eyes of Limulus. Application of either anoxia, DNP or 100% CO_2 causes, with a depolarization of 5 to 30 mV, a gradual reduction and eventually an abolition of the late receptor potential, and an increase in the latency and time to peak of the receptor potential. For example, in the lateral eye of Limulus, the latency at room temperature (23° C), measured by the time between the flash and 10% of the peak response, changed from 25 msec in normal oxygenated seawater, to 73 msec in anoxia, 90 msec in 0.05 mM DNP and 92 msec in 100% CO_2, with a reduction of the response in each case to approximately 40% of the normal peak response. The lengthening of the time scale caused by either anoxia, DNP or 100% CO_2, is exactly opposite from the response of photoreceptors that have been light adapted or injected with calcium where a loss in sensitivity is associated with a decrease in latency and time to peak. Because of this observed difference in time scale, the actions of anoxia, DNP or CO_2 can not be attributed merely to a decrease in regulation of intracellular free calcium. Their actions must also be exerted, either directly or indirectly, at some other point in the transduction process. This research supported in part by USPHS Fellowship EY05090-01 to R. C. Lantz.

W-AM-C4 POTASSIUM ION DEPENDENCE OF "ON" AND "OFF" RESPONSES IN VISUAL CELLS OF THE SCALLOP RETINA. M. Carter Cornwall and A.L.F. Gorman, Department of Physiology, Boston University School of Medicine, Boston, Mass. 02118.

In distal photoreceptor cells in the eye of the scallop Pecten irradians, an increase in light intensity causes membrane potential to hyperpolarize ("on" response) while a decrease in light intensity causes membrane potential to depolarize ("off" response). Similar "on" and "off" responses are elicited by different wavelengths of conditioning and background light. Experiments were designed to examine the role of potassium permeability changes in the generation of "on" and "off" responses. The retina was perfused with Na^+ free artificial seawater (choline substituted) to remove contributions of Na^+ ion and to displace membrane potential closer to the potassium equilibrium potential to facilitate reversal potential (E_r) determinations. E_r of the hyperpolarizing response to incremental changes in intensity of white light flashes, or blue light flashes on a red adapting background, and E_r for the depolarizing response associated with decrements in light intensity or red flashes on a blue adapting background are identical (-93.5 mV ± 2.6 mV s.d.). E_r was measured under all four light conditions as external potassium was varied between 9 mM and 100 mM. E_r changed 55 mV per decade change in external potassium, indicating that both "on" and "off" responses depend on changes in potassium permeability. Intracellular potassium concentration was calculated to be 387 mM. While previous experiments suggest that two pigment states are involved in generation of "on" and "off" responses in these cells, these data suggest that they are linked to the transduction process by a common ionic mechanism. This research supported by NIH Grant EY01157.

W-AM-C5 IDENTIFICATION OF A VITAMIN A COMPOUND IN EXTRACTS OF PIGMENTED GRANULES FROM APLYSIA NEURONS. J. M. Krauhs*, L.A. Sordahl, A.M. Brown, Dept. Physiology & Biophysics and Div. Biochemistry, University of Texas Medical Branch, Galveston, Texas 77550

The light-sensitive neurons of Aplysia have been investigated as photoreceptor models for decades, but the pigment(s) believed to be involved in the light response has seldom been studied. It is thought to be localized in membrane-bound organelles (lipochondria) which have been found to release Ca after exposure of the cells to light (Brown et al,1975, Science 188:157). A pure fraction of lipochondria was isolated from ganglia homogenates by centrifugation 3 times in a swinging bucket rotor at 100,000 x g. Electron microscopy showed this preparation to be free of all other organelles. Pigment was extracted from ganglia and from isolated lipochondria (LP) by homogenizing with chloroform-methanol, precipitating with 1% digitonin, solubilizing in 0.5% SDS, and extracting into chloroform. The extracts and standards were spotted on silanized silica gel plates for thin-layer chromatography (TLC) and developed in acetone or, for 2-dimensional TLC, acetone and 30% acetone in petroleum ether. The ganglia extract contained orange β-carotene, but its major component was a pale yellow, highly fluorescent (yellow-green) material which was identified as retinol or one of its derivatives. This was also the major component of the LP extract, which contained little or no β-carotene. The yellow pigment appeared to be associated with a protein, from which it could be separated with SDS. The LP of a dark-adapted R2 cell of the Aplysia abdominal ganglion were also highly fluorescent. It appears, therefore, that β-carotene, which dominates the visible light absorption spectrum of ganglia extracts, is present chiefly in the cytoplasm of the neurons. It may be a precursor for the synthesis of a "rhodopsin-like" compound which is the major pigment of LP. Supported by NIH grant NS-11453 and NIH Fellowship 1 F32 NS05508-01.

W-AM-C6 HALIDE CONTROL OF THE COLOR OF THE CONE VISUAL PIGMENT IODOPSIN. L. Y. Fager‡ and R. S. Fager, University of Virginia, Physiology Department , Charlottesville,Va. 22901

The chicken retina contains two visual pigments, rhodopsin, a typical rod pigment, and iodopsin, the best studied cone pigment. We have found both proteins to be glycoproteins and to have similar molecular weights, with iodopsin slightly smaller. In the photoreceptor membranes iodopsin has a spectral maximum at 562nm and rhodopsin has a maximum at 507nm; in detergents the same maxima are seen and presumably are those in the retina. Usual preparations of these membranes have contained several millimolar chloride ion. If chloride is rigorously removed, the spectrum of iodopsin shifts to 525nm. The shift is reversible; adding chloride back brings the spectrum to 562nm. The half point of titration is 1.7mM chloride. The positive counter ion of chloride has no effect. Bromide shows almost identical titration to an identical spectrum. Neither fluoride nor iodide have any effect. The phenomenon takes place either in detergent extracts or in the membrane. Rhodopsin's spectrum has no halide dependence. This phenomenon suggests, but does not prove, the existence of an anion binding site which direcrly interacts with the cone pigment chromophore.

W-AM-C7 CHROMATIC INTERACTIONS IN AMPHIBIAN PINEAL IN ABSENCE OF SODIUM IONS. W. Eldred* and J. Nolte*, (Intr. by R. Pannbacker), Anatomy Dept., University of Colorado Medical School, Denver, Colorado 80262.

The frontal organ, part of the anuran pineal complex, is photosensitive and gives excitatory and inhibitory electrical responses to green and ultraviolet (UV) stimuli respectively. A UV stimulus gives a long-lasting negative slow potential at the cut end of the pineal nerve, with long-lasting inhibition of the spike activity normally present in darkness, both of which can be reversed by a subsequent green stimulus. Since interactions take place between green and UV stimuli previous workers have suggested that separate green and UV-sensitive photoreceptors synapse on single ganglion cells. We have found, however, that these same interactions can take place in the absence of synaptic interactions, and probably in the absence of receptor potentials. When recording extracellularly from the pineal nerve in a Na^+-free medium (choline$^+$ substituted for Na^+) all action potentials and slow potentials in response to stimuli are abolished. If a frontal organ in Na^+-free Ringer is stimulated by UV, it remains inhibited for several minutes after returning to normal Ringer; this inhibition can be abolished by green light. If, however, in Na^+-free Ringer the UV stimulus is followed by a green stimulus, the preparation is not inhibited when returned to normal Ringer. The order of presentation of stimuli is important: if the UV stimulus follows a green stimulus in Na^+-free Ringer the preparation is inhibited after returning to normal Ringer. These interactions between stimuli in a Na^+-free medium suggest that neither synaptic activity nor receptor potentials are necessary for the interactions to take place. The interactions may originate in a light-induced interconversion of rhodopsin and a thermally stable photoproduct within single receptors.

W-AM-C8 INTENSITY SHARING, UPPER AND LOWER LIMIT AND GENETIC DEPENDENCE OF VISUAL PIGMENT ABSORPTION SPECTRA. M.L. Wolbarsht and B.S. Yamanashi, Duke University Eye Center, Durham, North Carolina 27710

A model is proposed for all vertebrate visual pigments (both rod and cone) in which the same chromophore (11-cis retinal or 3-dehydro retinal) is coupled to a variety of proteins via a Schiff base linkage. The major spectral characteristics of the photopigment depend on modifications of the electronic environment of the Schiff base nitrogen by the terminal groups of neighboring amino acids whose type and position are dependent upon the genetically coded structure of the protein. The principal deviations in the spectral absorption from Dartnall's nomogram depend upon intensity sharing between the alpha and beta bands. A_2 photopigments are the same as A_1 pigments except that the alpha band wavelength maximum of the A_2 type is displaced 20 nm towards the red. Calculations have been made to show that the major deviations from Dartnall's nomogram result from changes in intensity sharing from the symmetry dipole selection rule, especially when the chromophore is in the 11-cis configuration. Contributions from spin orbit and electronic vibrational coupling should be included and may explain aberrant absorption spectra such as iodopsin. A new nomogram has been constructed which gives a curve approximately invariant on a wavelength scale as contrasted with Dartnall's nomogram, which is invariant on a wave number scale.

W-AM-C9 TRANSMISSION SPECTRA OF TURTLE CONE OIL DROPLETS. L.E. Lipetz, Dept. of Biophysics, The Ohio State University, Columbus, Ohio 43210.

The oil droplets of Emys blandings have such high optical densities that their transmission spectra cannot be measured directly. Transmission spectra were measured from flattened droplets. Microspectrophotometry of intact droplets gave λ'_i, the intercept with the transmission minimum of the line tangent to the transmission curve at the point midway between minimum and 100% transmission. It was shown from optical relations that the approximate maximum optical density, Dmax, of a droplet could be calculated from its λ'_i and the flattened droplet's spectrum. Visual photometry confirmed that the results were correct within 20%. The yellow droplets had an absorption peak at 453.2 ± 1.1 nm, a lower peak at 481.7 ± 0.6 nm, and a shoulder at ~430 nm, matching the spectrum of zeaxanthin. The orange and the red droplets all had a single absorption peak at 478.2 ± 1.3 nm, matching astaxanthin. The clear droplets had transmission $\geq$0.94 for the range 390 to 750 nm. The regression line of Dmax vs droplet diameter, d, in micrometers was: for red, Dmax = 9.47 + 3.44d; for orange, Dmax = 1.52 + 0.99d; for yellow, Dmax = 4.00 + 0.23d. The regressions for red and orange were significant at the 5% level, that for yellow not even at the 20% level. Dmax ranged from 3.6 to 71 for red, 2.4 to 27 for orange, 1.5 to 9.6 for yellow. The ellipsoid of the accessory cone contained a light yellow pigment between the mitochondria. It had the same transmission spectrum as the yellow droplet, with Dmax $\simeq$ 1.

W-AM-C10 WATER-PHOTORECEPTOR MEMBRANE INTERACTION. D.S. Kirkpatrick[1] J.E. McGinness,*[2] P. Corry,*[2] W. Moorhead,*[3] H.M. Garza,*[1] and P.H. Proctor*[1]: (1) Dept. of Ophthalmology, Baylor College of Medicine, Houston, TX; (2) Dept. of Physics, Univ. of Texas Cancer Center, Houston, TX; (3) Dept. of Physics, Youngstown State Univ., Youngstown, OH.

Dielectric constant (μ), specific resistivity (ρ), and dielectric relaxation times (τ) of purified bovine rod outer segments (ROS) were measured as functions of temperature by time domain reflectometry[1]. τ for the major population of water associated with ROS became shorter as temperature decreased, while τ for controls, pure water and NaCl (0.1 M) became longer. Reversal of the temperature dependance of τ was previously observed for hydrated lysozyme powder[2]. ρ of ROS as well as of controls increased continuously as temperature decreased to the freezing point, where ρ increased sharply. As the temperature rose from the freezing point, ρ returned to prefreezing values along a different path, describing a hysteresis loop for ROS but not for controls. μ remained constant for both ROS and controls as temperature decreased to the freezing point where μ decreased sharply. As the temperature rose from the freezing point, μ returned to prefreezing values over a hysteresis path similar to ρ for ROS but not for controls. Both the reversal of the temperature dependance of τ of the major population of water associated with ROS, as well as the temperature hysteresis of τ, μ, and ρ demonstrated that a large fraction of the associated water interacted with the macromolecules of the photoreceptor membranes (surfaces). It has become apparent that the physical interpretation of these data requires understanding the detailed interactions which are quantum mechanical. *Supported by the Retina Research Foundation.*

1). A.Suggett *in* "New Techniques in Biophysics & Cell Biology"(1975),J.Wiley, p.191-232.
2). S.C.Harvey and P.Hoekstra (1972), J. Phys. Chem. *76*:2987-2994.

W-AM-C11 TRANSMISSION OF VISUAL INFORMATION BY THE LIMULUS EYE. F.A.Dodge and E. Kaplan,* IBM Research,Yorktown Heights,N.Y.10598 and The Rockefeller University,New York,N.Y. 10021

On the assumption that the lateral eye of Limulus is optimally designed to transmit information about its natural environment, we seek to discover its design principles by measuring what natural visual features are enhanced or suppressed in the neural response of this retina. On a sunny day the luminance of a sandy bottom 1.5 m below the surface of the somewhat murky water around the MBL, Woods Hole, is about 200 millilamberts. As a photodetector is rotated to the horizontal the light intensity falls by a factor typically more than 3, causing a steep gradient of the average excitation over the photoreceptor array. The automatic gain control of the generator potential mechanism effectively compensates the dc component of this gradient; and as previously shown (Biophys.J. 16:147a) lateral inhibition effectively suppresses the common-mode ac signal generated by the animal's motion in this gradient. The prominent visual feature of the sandy bottom is sand waves of about 10 cm period and peak to trough contrast of about 10%. A regular behavior of animals in this environment is "running" in a straight line at speeds of 10-25 cm/sec. (Invariably the animal turned away if, during this behavior, the experimenter approached within about 1 m.) In this environment the scanning of sand waves is thus a major ac component within the pass band of the photoreceptor dynamics. Since the inhibitory fields are uniform in the horizontal direction (Biophys.J. 15:172a) there exists, in principle, a translationly invariant neural response to horizontal scans at uniform velocity. Laboratory reconstruction of such "stationary" patterns from the spike-rate response to different scan rates can reveal the speed-dependence of visual feature enhancement.

W-AM-C12 RESPONSE CHARACTERISTICS OF AORTIC BARORECEPTORS ELICITED BY BROAD-BAND ("WHITE NOISE") PRESSURE STIMULI. F.H. Tuley, A.M. Brown, and W.R. Saum,* Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

The response of individual aortic baroreceptors to "white noise" pressure stimulus applied to the aortic arch have been examined in normotensive (NTR's) and spontaneously hypertensive rats (SHR's). The instantaneous frequency response using this technique was compared to results obtained from sinusoidal pressure inputs. The mean pressure levels for both techniques were essentially the same and covered the linear portions of the frequency-response curves. The frequency range for the "white noise" experiments was 0.01 to 50 and for the sinusoidal experiments was 0.01 to 20 Hz. The maximum peak to peak amplitude was 20 mm Hg. The impedance and phases of response computed for the "white noise" experiments using a Fast Fourier Transform program to obtain the transfer function were found to agree with results from the sinusoidal experiments. The results justify the use of the "white noise" technique for rapid accumulation of data for this preparation. The impedance which is related to receptor gain began to decline at 5 to 20 Hz for the receptors with myelinated axons and had a much smaller frequency range for the receptors with unmyelinated axons.

W-AM-C13 RESPONSES OF GOLGI TENDON ORGANS IN VITRO. Y. Fukami and R.S. Wilkinson, Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis Missouri 63110.

Responses to controlled stretch have been studied in isolated Golgi tendon organs (GTOs) from tail muscles of cat. Isolated GTOs were mounted in a chamber, one end tied to a rod connected to a tension transducer, the other to an electromechanical device for stretching. The isolated axon was drawn into oil for recording. The threshold force producing sustained discharge at 24°C ranged from 8 to 170 dynes in 9 preparations. Beyond threshold, static frequency was approximately proportional to applied tension. The elastic modulus of the GTOs ranged from 1.9×10^7 to 3.5×10^8 dynes/cm^2, a value similar to that found for ordinary cat tail tendons. Sensitivity (impulses/sec/unit stress) was inversely proportional to elastic modulus suggesting that the afferent response is principally determined by strain. With impulse activity blocked by tetrodotoxin, graded receptor potentials were recorded whose amplitude varied with tension. All GTOs examined showed a dynamic response, which became larger with increasing stretch velocity. This was evident in both the receptor potential and the afferent discharge.

W-AM-C14 IONIC BASIS OF RECEPTOR POTENTIAL IN ISOLATED MAMMALIAN MUSCLE SPINDLES. C.C. Hunt, Y. Fukami and R.S. Wilkinson, Dept. Physiol. and Biophysics, Washington Univ. School of Medicine, St. Louis, Mo. 63110.

Removal of the capsule from the sensory region of cat muscle spindles allows rapid exchange of bathing fluid with the cleft between sensory terminals and intrafusal fibers, as shown by lanthanum staining in electron micrographs. Such isolated spindles were mounted in a rapid flow chamber, the poles tied to Brush pen motors, and the primary axon drawn on a recording pipette into heavy oil. After impulse block by TTX in normal Locke solution, ramp-and-hold stretch evokes a depolarization, the receptor potential, with a large dynamic and smaller static component. On release from stretch the ending becomes hyperpolarized; there is also a tendency to undershoot after the dynamic response to high velocity stretch. Solutions containing TRIS, choline, or glucosamine in place of Na^+ and Ca^{2+} reduced the receptor potential within ca 20 sec to less than 1/5 its control value. The response was gradually abolished over 20-30 min. Addition of 1.8 mM Ca^{2+} partly restored response. Substitution of isethionate or methylsulfate for Cl^- produced no significant change in receptor potential. Depolarization produced by stretch appears due to an increase in conductance to Na^+ and, to a lesser extent, to Ca^{2+}. Post-dynamic and release hyperpolarization increase relative to baseline level as background stretch is increased; they are diminished as $[K^+]_o$ is raised, suggesting an increase in potassium conductance is responsible. These effects may be voltage dependent.

W-AM-C15 NOISE SPECTRA FROM HAIR CELLS OF THE *HERMISSENDA* STATOCYST. L.J. DeFelice and D.L. Alkon, Anatomy Dept., Emory Univ.,Atlanta GA 30322 and NINCDS, NIH, Lab. of Biophys., MBL., Woods Hole, Mass. 02543.

Voltage noise was measured from hair cells during physiological stimulation. Microelectrodes penetrated the soma of hair cells with cut and intact axons. Spontaneous firing was suppressed by hyperpolarizing the soma under current clamp. Spontaneous voltage fluctuations were measured at mean membrane potentials between -60 and -200 mV. The preparation was located 19 cm from the center of rotation. At a fixed potential, the preparation was rotated between 0 - 1.6 rps. Noise was measured during constant rotational velocity. Cells in front of the force vector depolarize and voltage noise increases. Behind the vector, cells hyperpolarize and voltage noise decreases. A 1 mV change in mean membrane potential is accompanied by an average rms change in noise of 110 μV. Changes between 1 - 30 mV were achieved by rotation. Stopping the rotation causes a brief overshoot followed by a slow recovery to control levels. Power spectra S(f) fit a relaxation (τ) spectrum at low frequencies. At -80 mV, a 6 mV hyperpolarizing response decreases S(0) from 5.9 to $4.3 \times 10^{-9} V^2/Hz$, and increases τ from 65 to 166 msec. At a constant rotational velocity, noise power increases with increased hyperpolarizing current. At a constant current, noise power changes are proportional to rotational speed. The data are consistent with a shot model of statoconia-hair cell interaction. At -80 mV, the average interaction between statoconia and hairs causes a 20 μV change in potential. For an 80 MΩ input resistance, this corresponds to a brief current of 2.5×10^{-13}A. The exact driving force for this current is unknown. A crude estimate of the elemental conductance change is $10^{-11}\Omega^{-1}$.

W-AM-C16 CD AND FLUORESCENCE EVIDENCE FOR CONFORMATIONAL CHANGES IN GALACTOSE AND RIBOSE RECEPTORS OF S TYPHIMURIMUM. P.R. Hartig, R.S. Zukin, D.E. Koshland, Jr., Laboratory of Chemical Biodynamics and Department of Chemistry, University of California, Berkeley, 94720.

The CD spectrum of purified S. Typhimurium ribose receptor reveals a broad positive peak between 270 and 290 nm and sharp negative peaks at 261 and 268 nm. Addition of saturating ribose causes a broad CD decrease between 270 and 290 nm assignable to tyrosine residues (cystine and tryptophan are not present). Addition of saturating ribose also induces a sharp CD decrease at 262 nm assignable to phenylalanine residues. Addition of nonbinding sucrose caused no detectable CD changes. 5-Iodoacetamidofluorescein (5-IAF) labeled galactose receptor shows fluorescence changes on galactose binding. The presence of spectral changes at several sites suggests that ligand binding induces delocalized conformational changes. Galactose receptor contains a single tryptophan and can be labeled by 5-IAF at a single residue. Fluorescence lifetime, fluorescence emission and KI quenching studies reveal significant changes in both tryptophan and 5-IAF fluorescence upon galactose addition. Energy transfer from tryp to 5-IAF was measured and a modification of the theory of Dale and Eisinger (Biopolymers 13, 1573 (1974)) for an ellipsoidal protein was developed to determine the range of K^2 values. A minimum tyr to 5-IAF separation of 33Å was obtained. The existence of spectral changes at two widely separated residues on the galactose receptor upon galactose binding at a single site reveals the presence of a delocalized ligand-induced conformational change. These induced conformational changes may serve to initiate the interaction of the sugar receptors with a membrane bound signaling component, thus triggering the chemotactic response (Strange and Koshland, PNAS 73, 762 (76)).

W-AM-D1 EFFECT OF NYSTATIN ON THE ELECTRICAL PROPERTIES OF RABBIT URINARY BLADDER. S.A. Lewis, D.C. Eaton, C. Clausen[1], and J.M. Diamond[1]. Dept. of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550. [1]Dept. of Physiology, U.C.L.A. Medical School, Los Angeles, California 90024.

The polyene antibiotic nystatin decreased the transepithelial resistance (R) of rabbit urinary bladder when applied to the mucosal solution. The transepithelial potential (V) was either increased or decreased to a common value of -50 mV (with respect to the serosal solution). Using microelectrodes, we studied the location of the R decrease in response to nystatin addition to the mucosal solution. It was found that nystatin decreased the resistance of only the apical membrane (R_a). The degree of reversal of the nystatin induced R decrease was dependent on the composition of the mucosal bathing solution. When the mucosal bathing solution was NaCl - $NaHCO_3$ Ringers + nystatin, cell swelling and desquamation was observed, and R continually decreased. However, when the mucosal bathing solution was KCl - $KHCO_3$ Ringers + nystatin, there was no cell swelling and R reached a steady state value which was approximately equal to the basolateral membrane resistance (R_{bl}), indicating that nystatin was not inserting itself into the basolateral membrane.

W-AM-D2 AC IMPEDANCE ANALYSES IN A TIGHT EPITHELIUM: LIMITATIONS OF THE METHOD AND INITIAL RESULTS. C. Clausen, S.A. Lewis‡ J.M. Diamond, and D.C. Eaton‡ UCLA Dept. of Physiology, Los Angeles, CA 90024 and ‡Dept. of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

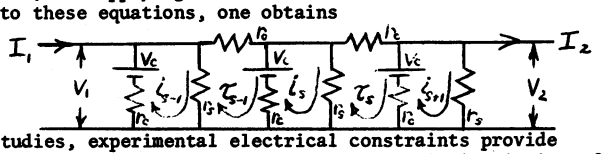
In an attempt to ascertain apical, basolateral, and junctional conductances as well as estimate apical and basolateral membrane areas by measuring those membranes' capacitances, equivalent circuit analyses were carried out in the tight epithelium, rabbit urinary bladder. The goal of the analyses was to determine the membrane conductances per unit area by normalizing the measured conductances to the respective membrane capacitances. The method consists of fitting transepithelial impedance measured as a function of frequency to a morphologically-based equivalent circuit made up of resistors and capacitors. Some limitations of the method are: (1) microelectrodes must be used to independently measure the ratio of the apical to basolateral membrane resistances (voltage divider ratio); (2) the lumped model, a simple parallel resistor-capacitor combination, is not adequate to model the basolateral membrane; (3) under conditions where the total DC transepithelial resistance is low (e.g., high transport or applied nystatin) the AC method fails due to the added impedance of the lower two cell layers and possibly connective tissue. The method has provided us with the following information: (1) unless the preparation is highly stretched, the small geometry of the lateral intercellular spaces requires the use of a more complicated model than the commonly used lumped model; (2) the basolateral membrane resistance is some 2 to 3 times lower than was previously reported, and (3) there is an increase in apical capacitance (i.e., surface area) with time (possibly caused by the degree of stretch of the preparation), a result of considerable importance when interpreting conductance changes in the preparation.

W-AM-D3 ELECTROPHYSIOLOGY OF EPITHELIAL CELL LAYERS-A FINITE DIFFERENCE MODEL. C.J.Myers* and W.McD.Armstrong, Department of Physiology, Indiana University School of Medicine, Indianapolis, Indiana 46202

Epithelial cell layers may be electrically modeled as a ladder network composed of periodically repeating discrete circuit elements. Cable analysis of such models (e.g. Shiba, J. Theoret. Biol. 30:59,1971) is unsatisfactory because it does not formally allow the existence of separate transcellular and paracellular conducting pathways nor the inclusion of transepithelial potential sources. A finite difference analysis of the ladder network shown permits the inclusion of these parameters as discrete model elements. r_c, r_s and r_o are the transcellular, shunt, and bathing fluid resistances. i_s and τ_s are mesh currents. V_c is the transepithelial potential. Using Kirchhoff's voltage law to write mesh equations for the s'th loop set, and applying forward and inverse difference calculus operators (E, E^{-1}; e.g. $Ei_s = i_{s+1}$) to these equations, one obtains

$$-(r_s+r_c)i_s+(r_s+r_cE^{-1})\tau_s+V_c=0$$

$$(r_s+r_cE)i_s-(r_s+r_o+r_c)\tau_s-V_c=0$$



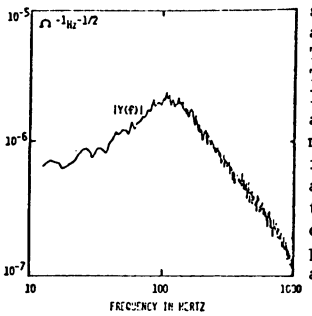
In applying the model to tissue studies, experimental electrical constraints provide boundary conditions. Coupled to the above equations, these permit numerical solutions for r_c, r_s and V_c in terms of the experimentally established parameters I_1, V_1; I_2, V_2 and r_o at the periphery of the tissue. The use of this model to determine r_s in isolated bullfrog small intestine will be illustrated. (Supported by USPHS Grants AM 12715, HL 06308).

W-AM-D4 ANALOGUE SOLUTION FOR ELECTRICAL CAPACITY OF MEMBRANE COVERED CUBES IN CUBIC ARRAY AT HIGH CONCENTRATION. K.S.Cole, Laboratory of Biophysics, NINCDS, National Institutes of Health, Bethesda, Md. 20014 and MBL, Woods Hole, Massachusetts 02543.

Earlier measurements have shown that the equations derived for the resistance of a suspension of spheres or cylinders apply up to a volume concentration of 100% for close packing forms. They have also shown, by a resistance-capacitance analogue, that they apply for the capacity of a close packing of membrane covered square cylinders in square array, approximating muscle and nerve. The present work is an extension of the two-dimensional to a three-dimensional array of membrane covered cubes in a cubic array. It is found by measurements of a three-dimensional electrolytic analogue that the capacitance is indeed expressed by an extension of the analytic solution at low concentrations up to 100% volume concentration of the membrane covered form, such as epithelial tissues. There is thus at least one example each of two and three dimensional forms which conform to the low concentration analysis up to 100% volume and so give a basis for the extensions to other and more complicated forms to complete a survey of the work begun by Fricke in 1923.

W-AM-D5 EQUIVALENT INDUCTANCE IN FROG SKIN PATCH CLAMP CURRENT NOISE. T. Hoshiko and J.R. Clarke*, Dept. of Physiology, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106

Previous work (JGP66:16a,1975) on clamp current fluctuations in 0.07 cm^2 area of frog skin showed a power density spectrum (PDS) dominated by low frequency components. Use of a double pipette patch clamp of 0.001 cm^2 under a high density fluorosilicone oil has revealed much higher frequency components, and actually a well defined peak in the current PDS. The admittance spectrum matches the peak in the current PDS. In some skins, no peak was apparent but the break in the curve was too sharp to be Lorentzian. The final limiting slope in all instances approached $1/f^2$. The resonance peak disappeared on substitution of K^+ for Na^+ in the patch pipette. The peak has been observed in both chloride and sulfate Ringer's. The resonance frequencies observed varied from 100 to 700 Hz. In some instances, the peak shifted as the clamp potential was altered. The rise in amplified input voltage noise occurred at much higher frequencies in the clamp-amplifier combination used in these experiments. Gross mechanical vibration effects were also ruled out as an explanation of the peak. In the case of the admittance peak illustrated, assuming a simple RLC series circuit, for the resonance frequency of 118 Hz, and half power points, R_o calculates to be 4.5 x 10^{-2} Ω cm^2, L = 0.76 henry-cm^2 and c = 2.4 x 10^{-6} f cm^{-2}. Supported by USPHS.



W-AM-D6 In Vitro FORMATION OF CELL LAYERS WITH PROPERTIES OF EPITHELIAL MEMBRANES. M. Cereijido, C.A. Rotunno, E.S. Robbins and D.D. Sabatini. Dept. of Cell Biology N.Y.U. Medical Center, 550 First Ave. New York, N. Y. 10016.

This work describes a technique for the preparation of epithelial cell layers on a permeable and transluscent support, and illustrates its use to study the formation and characterization of tight junctions. Collagen coated discs (1-2 cm in diameter) of a fine mesh nylon cloth were used. Several cell lines were tested: W138, BSC-1, BHK, SIRC, MDBK and MDCK. The cells were plated from suspension densities high enough (2x10^6 cells/cm^2) to saturate the area available for growth. 90 min. after plating the discs were transferred to a Petri dish with fresh medium and kept in an 5% CO_2 incubator with constant humidity. These discs were later mounted as flat sheets between Lucite chambers to test membrane properties. When the preparation is made with MDCK cells (epithelial-like derived from a dog's kidney) the maximum transepithelial resistance (150 to 250 Ω cm^{-1}) is achieved 14 to 18 hours after plating. This value then decreases and remains constant from 1 to 30 days: 84.2 ± 2.9 (44) Ω cm^2. The time course of the increase in resistance is taken to reflect the formation of tight junctions. Inhibitors of protein sinthesis (Puromycin or cycloheximide), if present before the 5th hour of plating, inhibit reversibly the development of resistance. Inhibitors of the synthesis of RNA (DRB) do not. The Na to Cl permeability ratio (β) is 8.3 in steady state. The order of cation selectivity is K>Na>Rb>Cs>Li. The establishment of β does not follow the same time course as the increase of resistance, suggesting that junctions undergo a process of maturation. The removal of Ca^{++} and addition of EGTA decreases the resistance in a few seconds to a half its control value and to less of 5% in one hour. Readmission of Ca^{++} produces a complete recovery of the resistance. (supported by USPHS Grant AG 00378).

W-AM-D7 WEAK ELECTROLYTE INFLUX IN RAT SMALL INTESTINE. M.J. Jackson, A.M. Williamson*, W.A. Dombrowski* and D.E. Garner* Physiology Department, George Washington University Medical Center, Washington DC 20037.

Hogben et al [J.Pharmacol. 125,275(1959)] proposed that the transport of weak electrolytes in rat small intestine is influenced by the presence of a low pH microclimate at the luminal surface of the epithelial cells. We have tested this hypothesis by measuring the unidirectional influxes of 22 weak acids and 9 weak bases into segments of jejunum and ileum incubated in vitro. Preliminary experiments showed that influx increased linearly with time and concentration, and varied with pH in a manner suggestive of non-ionic diffusion modified by the presence of an unstirred layer. Calculation showed that the unstirred layer was not rate limiting in these experiments, and that the influxes of a weak acid and a comparably permeant weak base should differ by as much as four orders of magnitude if the pH at the luminal cell surface was two units lower than that of the bulk phase. The influxes were linearly related with partition coefficients in two phase organic solvent/aqueous systems, and the lines describing these relations for weak acids and weak bases did not differ significantly. We conclude that we have been unable to demonstrate the existence of a functional low pH microclimate at the luminal surface of intestinal epithelial cells, and that the driving force for weak electrolyte transport in the intestine cannot be described in terms of a difference between the pH values of the luminal surface layers and the bulk phase.

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W-AM-D8 DIFFUSION OF XENON THROUGH EPITHELIA AND THROUGH WATER BOUNDARY LAYERS. Gerald L. Pollack, Physics Department, Michigan State University, East Lansing Michigan. 48824

Xenon and the other rare gases are comparatively inert yet undergo interesting biological interactions. Some applications of these interactions are to: general anesthetics, deep sea diving physiology, nuclear medicine, and nuclear reactor safety. Transport of these elements through epithelial tissue is a key part of several of the interactions. We have measured the total diffusion constant d as a function of stirring frequency ω for Xe-133 through frog skin and toad bladder epithelia. The diffusion constants for the frog skins and toad bladders are, respectively, $d_m = (3.9 \pm 0.8) \times 10^{-4}$ cm/sec and $(7.4 \pm 4.2) \times 10^{-4}$ cm/sec. "Unstirred" water layer thickness δ is determined concurrently from the frequency dependence of $d(\omega)$ using a new method suggested by hydrodynamic first principles. The result for frog skin is $\delta = (0.060 \pm 0.016)/\sqrt{\omega}$(rad/sec) cm. The stirring frequency range is from ω = 7.5 rad/sec (72 rev/min) to 55 rad/sec (530 rev/min). The results support the conclusions that the principal barrier to Xe diffusion in these epithelia is inter- and intracellular water, and that the diffusion is passive and rapid. The experimental method may be straightforwardly adapted to the measurement of diffusion, or counterdiffusion, of any gamma radioactive, soluble or partly soluble, solute through any flat membrane or through a solvent. We estimate the amount of total body absorbed radioactivity due to environmental Xe-133 to be 50 femtoCi for an ambient concentration of 2.6 picoCi/m^3 of air. The analogous quantity for Kr-85 is 18 picoCi, assuming an ambient concentration of Kr-85 of 3nanoCi/m^3.

Supported by E.R.D.A. Grant AT(11-1)-1574.

W-AM-D9 INTRACELLULAR CALCIUM REGULATES TRANSEPITHELIAL SODIUM TRANSPORT IN THE FROG SKIN. D. Erlij and S. Grinstein*. Dept. of Physiol., Centro de Investigacion, IPN, Mexico, D.F. and Dept. of Physiol., SUNY, Downstate Medical Center, Brooklyn, New York 11203.

Exposure of frog skins to Na-free solutions on the basal border causes a reduction in transepithelial Na transport. Since Na-free solutions produce an increase in cell Ca concentration in some cell types, we have explored whether such increase plays a role in their inhibitory effects on transepithelial transport. Measurements of ^{45}Ca uptake in isolated epithelia showed that Na-free solutions caused a threefold stimulation of Ca uptake; nearly all the increased uptake occurred through the basal border. The reduction in transepithelial Na transport caused by Na-free solutions was absent when Ca is also eliminated from the basal solution. These findings suggest that the reduction in transepithelial Na transport observed when the basal border of the epithelium is exposed to Na-free solutions is caused by increased cytoplasmic calcium levels. We also found that during the action of metabolic inhibitors, Ca efflux from the epithelium was markedly increased. Since such an increase in efflux is probably due to increased [Ca] in the cytoplasm we suggest that the reduced epithelial Na permeability observed during metabolic inhibition is due to increased cytoplasmic calcium. If this conclusion is correct, Ca ions could couple the level of the rate of transport to the level of energy metabolism.

W-AM-D10 CELLULAR MECHANISMS UNDERLYING THE EPINEPHRINE STIMULATION OF Cl SECRETION BY THE RABBIT CORNEAL EPITHELIUM. R.K.S. Wong* and S.D. Klyce, Div. Ophthalmology, Dept. Surgery, Stanford Medical School, Stanford, CA 94305.

Epinephrine increases corneal cyclic AMP production and stimulates epithelial Cl secretion in vitro. Intracellular records indicate that pulses of epinephrine ($5x10^{-10}$ M) delivered to the tear or stromal side of the epithelium transiently decrease its resistance through action on the outer, tear-facing cellular membrane. Neither the stromal-facing membrane nor the paracellular pathway resistances were significantly affected. Small changes in epithelial potential during this response also occurred at the outer cellular membrane. The magnitude and polarity of the potential response was dependent on tear [Cl] and on the prestimulus epithelial potential. Outer cellular membrane reversal potentials for the response were determined at tear [Cl] ranging from 100 mM to 21 mM, and a semilogarithmic function was found with a slope of 56.2 mV/decade, close to the Nernst equation prediction of 61 mV/decade. This, along with other evidence, suggests that epinephrine specifically increases the Cl conductance of the membrane. From the Cl electrode behavior, cell [Cl] was calculated to be 41.5 mM. The uptake of Cl from the stroma to the cells following its depletion was inhibited 84% by 10^{-5} M ouabain.

Cl secretion in this tissue is apparently accomplished by active transport into the cells from the stroma, a process which is in series with a diffusive movement from the cells to the tears across the outer cellular membrane. Epinephrine modulates the latter movement via cyclic AMP.

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W-AM-D11 ANION TRANSPORT IN SITS-TREATED TURTLE BLADDERS. G. Ehrenspeck, W.A. Brodsky and J. Durham*, Department of Physiology, Division of Biophysics, Mount Sinai School of Medicine/CUNY, New York, New York 10029

Serosally-applied disulfonic stilbenes (SITS or DIDS) reduce the PD and I_{sc} by 100% and the net chloride reabsorption by 60% to 80% across turtle bladders in Na-free media with exogenous bicarbonate. Without exogenous bicarbonate, net chloride reabsorption is not detectable and SITS reduces PD and I_{sc} by 100% and mucosal acidification by 75%. The coexistence of a zero PD and a finite reabsorption of chloride ion after SITS requires the simultaneous secretion of a negative ion other than chloride, probably bicarbonate. Although DIDS or SITS migrate slowly across the entire epithelium, little if any enters the cytoplasm. Consequently (Na + K)·ATPase activity and Na transport remain constant while anion transport is inhibited. Present data are consistent with (a) an "all-electrogenic" bladder with discrete pump-containing paths to accomodate the transepithelial flows of HCO_3 and Cl or (b) a "part-electrogenic" bladder containing an electrogenic acidification pump in the apical membrane and a neutral $Cl:HCO_3$ exchanger in the basal-lateral membrane. This study was supported by grants from the National Institutes of Health (AM 16928-03) and the National Science Foundation (PCM76-02344).

W-AM-D12 EFFECTS OF INHIBITORS OF Na AND Cl TRANSPORT ON OXYGEN CONSUMPTION IN THE BULLFROG CORNEA. P.S. Reinach* and H.F. Schoen* and (Intr. by I.L.Schwartz),Ophthalmology Dept. Mount Sinai School of Medicine of CUNY, New York, N.Y. 10029.

The oxygen consumption of isolated bullfrog corneas in NaCl, Na_2SO_4 (Cl-free) and choline Cl (Na-free) Ringer's solution was studied and also the effects on oxygen consumption of the inhibitors of Cl transport: furosemide, ethacrynic and dihydroethacrynic acids. The removal of Cl from the bathing medium inhibited oxygen consumption by 19% while its addition to a Cl-free medium stimulated oxygen consumption by 14%. Na removal from the bathing medium inhibited oxygen consumption by 36%. Furosemide inhibited oxygen consumption (16%) only in NaCl Ringer's solution. Ethacrynic and dihydroethacrynic acids inhibited oxygen consumption in NaCl and Cl-free media but their effects were less specific. The inhibitors of Na and Cl transport: ouabain and tryptamine, inhibited oxygen consumption in NaCl Ringer's solution by 34% and 26%, respectively. These results are pertinent for characterizing the coupling between aerobic metabolism and transepithelial active transport of Na and Cl. The fractions of suprabasal oxygen consumption supporting Na and Cl transport are found to be nearly equal while the rate of net transepithelial Cl transport is known to be at least four times as large as the rate of net active Na transport. Based on the furosemide effect on oxygen consumption and the electrical energy dissipation through the active Cl transport pathway, an energy efficiency utilization by Cl transport of about 50% is calculated.

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W-AM-D13 THE METABOLIC COST OF Na-TRANSPORT IN TOAD BLADDER. P. Labarca*, M. Canessa and A. Leaf. Laboratory of Renal Biophysics, Mass. General Hospital, Boston, Mass. 02114

The metabolic cost of active Na-transport was determined in toad bladders at different gradients of transepithelial potential, $\Delta\psi$, by continuous and simultaneous measurements of CO_2 production and of transepithelial current. Amiloride was used to block active Na-transport in order to assess the passive permeability of the tissue and the non-transport-linked (basal) production of CO_2. From these determinations, active Na-transport (J_{Na}) and suprabasal CO_2 production ($J^{sb}_{CO_2}$) were calculated. Since large transients in J_{Na} and $J^{sb}_{CO_2}$ frequently accompanied any abrupt change in $\Delta\psi$, steady state conditions were carefully defined. Some 20-40 minutes were required after a change in $\Delta\psi$ of $\pm$ 50 mV, before steady state of transport activity and of respiration were achieved. The metabolic cost of Na-transport proved to be the same whether the bladder expended energy moving Na against a potential of +50 mV or whether Na was being pulled through the active transport pathway by an electrical gradient of -50 mV. The ratio $J_{Na}/J^{sb}_{CO_2}$ (moles/moles) were 19.8 $\pm$ 1.6 at +50 mV, 21.7 $\pm$ 1.8 at -50 mV and 19.2 $\pm$ 0.9 at 0 mV. Comparison of this ratio calculated for the total 60 min period and for the last 10 minutes of the hour showed no significant differences, indicating that the early transients of J_{Na} and $J^{sb}_{CO_2}$ are compensated later when steady state conditions were achieved. The perturbation of $\Delta\psi$ did not elicit changes of J_{Na} nor, consequently, of $J^{sb}_{CO_2}$ by blocking the pump with 10^{-2}M ouabain. The independence of the ratio $J_{Na}/J^{sb}_{CO_2}$ over the range $\pm$ 50 mV indicates a high degree of coupling between active Na-transport and respiration. Thus, the data demonstrate that the ratio of Na actively transported to CO_2 produced is independent of the amount of work to be done in the transepithelial movement of Na across the toad bladder. Supported by grants: USPHS HE 06664 and NHLI RR 05486.

W-AM-D14 EFFECT OF AMINOPYRINE ON H^+ SECRETION AND THIOCYANATE INHIBITION OF IN VITRO FROG (Rana pipiens) GASTRIC MUCOSA. S. S. Sanders, J. A. Pirkle*, R. L. Shoemaker, and W. S. Rehm. Dept. of Physiology and Biophysics, University of Alabama in Birmingham, Birmingham, Alabama 35294

We have previously reported (Physiologist 19:350,364, 1976) that imidazole (IZ) a weak base (pK_a = 7.0) a) does not inhibit H^+ secretion (pH stat method) in the in vitro frog gastric mucosa but diffuses across from nutrient to secretory side in neutral form and neutralizes secreted H^+ ions, and b) reverses thiocyanate (SCN^-) inhibition of H^+ secretion. The effects of aminopyrine (AP) another weak base (pK_a = 5.0) were determined. When pH of secretory (sec) fluid = 4.0, AP decreases measured rate but upon increasing pH of sec from 4.0 to 6.0 the measured rate is restored demonstrating that mechanism is one of neutralization, i.e., the same as IZ. This effect of AP was predicted but we assumed that the reversal of SCN^- inhibition by IZ was specific for IZ. However we found that AP also reverses SCN^- inhibition. In Cl^- media with sec pH = 6.0, SCN^- reduces H^+ rate to zero and AP (20 to 30 mM) restores H^+ rate. Restored rate is reduced to zero by anoxia or amytal (8 mM). In Cl^--free media ($SO_4^=$ for Cl^-) using magnitude of inverted PD as criterion for H^+ secretion AP does not inhibit H^+ secretion and does reverse SCN^- inhibition. The fact that SCN^- inhibition is reversed by both weak bases gives us a lead towards the challenging problem of the mechanism of SCN^- inhibition which in turn should throw considerable light on the basic mechanism of H^+ production. NIH and NSF support.

W-AM-D15 ENERGIZED GASTRIC MICROSOMAL MEMBRANE VESICLES--AN INDEX USING METACHROMATIC DYES. H.C. Lee* and J.G. Forte, Dept. of Physiology-Anatomy, Univ. of California, Berkeley, Ca. 94720

Microsomal vesicles isolated from gastric mucosa possess a unique K^+-ATPase and are able to accumulate H^+ upon energization with ATP. In order to gain further insight into the mechanism of the H^+-pump, two metachromatic dyes, acridine orange and neutral red, were used as molecular probes. These dyes have the property that when binding to polyanion they exhibit a spectral absorption shift from monomer spectrum to that of dimer and higher aggregates. On addition of ATP, in the presence of K^+ and Mg^{2+}, dramatic dye uptake by hog gastric microsomal vesicles occurs. The absorption spectrum of the dye shows a metachromatic shift similar to that observed for binding to polyanion. This shift has the following properties: (1) requires both Mg^{2+} and K^+; (2) inhibited by K^+-ATPase inhibitors such as Zn^{2+} and F^-; (3) specific for ATP in the sense that ITP, GTP, pNPP cannot substitute; (4) reversed by nigericin alone or valinomycin and CCCP together; (5) occurs in a pH range from 5 to 8 with an optimum around pH 7.0; (6) kinetically corresponds closely to the H^+ uptake by these vesicles. Artificially imposed H^+-gradients from inside/out across vesicles produced a similar dye spectral shift. The magnitude is a function of Δ pH and is dissipated by proton ionophores. The data may be explained by the hypothesis that upon energization with ATP or pH-gradient a conformational change occurs exposing negative sites which is monitored by the spectral changes in these metachromatic dyes. (Supported by USPHS grant AM 10141.)

W-AM-D16 LOCALIZATION OF H^+:K^+ ATPase IN GASTRIC MUCOSA BY ANTIBODY TECHNIQUES.
G. Saccomani, A. Mihas*, S. Goodson*, and G. Sachs, Laboratory of Membrane Biology, University of Alabama in Birmingham, Birmingham, Alabama 35294

An H^+ transporting vesicular membrane fraction of high specific K^+-ATPase activity (100-110 μ moles/mg/hr) was isolated from hog gastric mucosa using differential and zonal density gradient centrifugation and free-flow electrophoresis. Antibodies were raised against a suspension of this purified membrane fraction in six New Zealand white rabbits. Purified γ-globulin from immunized animals, but not from control animals or preimmune serum from the experimental group, inhibited the K^+-ATPase activity up to 80% and the K^+-stimulated p-nitrophenylphosphatase by up to 35% in a concentration--dependent manner, but had no effect on the Mg^{2+}-ATPase activity. These antibodies also produced some degree of inhibition (∿30%) on the ATP dependent H^+ transport. Immunodiffusion of antisera against Triton X-100 solubilized enzyme showed a precipitation band whose intensity paralleled the degree of inhibition of the enzyme. The antigen-antibody complexes were localized in the gastric mucosa by indirect immunofluorescent staining. Antibody experiments using fundic sections demonstrated specific binding to the parietal cells especially in the apical region. Controls using preimmune serum or antral sections showed no reactivity. This suggests that the H^+:K^+ ATPase vesicles derived from the apical region of the gastric parietal cells. (NIH, NSF support).

W-AM-E1 COVALENT LABELING STUDIES OF PROTEINS ASSOCIATED WITH THE INNER SURFACE OF THE SQUID GIANT AXON MEMBRANE. H.C. Pant, T. Yoshioka*, J. Baumgold*, G. Matsumoto*, and I. Tasaki, Laboratory of Neurobiology, National Institute of Mental Health, Bethesda, Maryland 20014.

Proteins associated with the inner layers of squid giant axon membrane were labeled with iodinated (I^{125}) p-hydroxylphenyl propionic acid n-hydroxysuccinimide ester (so-called Bolton-Hunter reagent) which acylates terminal amino groups with the iodinated p-hydroxyphenyl propionic residue. Proteins were labeled inside excitable axons by internal perfusion or by adding Bolton-Hunter reagent to the perfusate *in vitro*. The labeled proteins were separated by polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS). The effects of pronase and of various chaotropic agents on the labeling of the axonal proteins were also studied. The results showed that the molecular weight profile of proteins in the perfusate is strongly influenced by these agents. The significance of these findings will be discussed.

W-AM-E2 INHIBITION OF Cl^- BINDING TO ANION TRANSPORT PROTEIN OF THE RED BLOOD CELL BY DIDS (4,4′-DIISOTHIOCYANO-2,2′-STILBENE DISULFONIC ACID) MEASURED BY ^{35}Cl NMR. Y. Shami, J. Carver, S. Ship* and A. Rothstein, Research Institute, The Hospital for Sick Children, and Dept. of Cell Biology, University of Toronto (J.C.), Toronto, Ontario.

Kinetic data suggest that chloride binding to membrane sites precedes its translocation. Demonstration of such binding by conventional methods is, however, difficult because the apparent affinity of the Cl^--site interaction is very low. The difficulty can be overcome by using ^{35}Cl NMR, because chloride relaxation times is sensitive to the presence of even low affinity anion binding sites. The width (at half height) of the NMR spectral peak of ^{35}Cl was increased significantly in the presence of red blood cell ghosts, indicating that the membranes bind chloride. The specific widening was a linear function of the quantity of ghosts, amounting to 4.2 Hz/mg of ghost protein. If cells were treated with the irreversible anion transport inhibitor, DIDS, prior to their preparation, the widening of the NMR peak was substantially diminished (by 32%), indicating that DIDS reduces Cl^--binding. Because DIDS is highly localized in a particular protein (band 3), Triton X-100 extracts enriched in this protein were also tested. The peak widening in this case was 6 Hz/mg of protein, greater than that observed per mg of ghost protein. The widening was reduced by almost 50% if the proteins were extracted from cells treated with DIDS, with the effect of the agent largely limited to the pH range 6.5 to 8.5. The data suggest that the effectiveness of DIDS as an inhibitor of anion transport may be related to its capacity to displace Cl^- from anion binding sites in band 3. (This study was supported by a Medical Research Council (Canada) Grant # MT 4665)

W-AM-E3 COMMUNICATION AMONG CELLULAR GLYCOLYTIC ENZYMES: ^{31}P NMR EVIDENCE FROM OUABAIN ADDITION TO RED CELL MEMBRANES E.T. Fossel* and A.K. Solomon, Biophysical Laboratory and Department of Biological Chemistry, Harvard Medical School, Boston, Mass. 02115

Addition of 10^{-6} M ouabain to human red cells, depleted of glucose for 2 hr at 37°C, shifts the ^{31}P nuclear magnetic resonance peaks of cell 2,3-diphosphoglycerate (2,3-DPG). Fossel and Solomon (Biophys. Biochim. Acta, in press) have added monophosphoglycerate mutase (MPGM) to inside out vesicles to show that this resonance shift results from binding by MPGM of its cofactor, 2,3-DPG. At 24.15 MHz, the shift is 1.6 Hz (3-phosphate; 2-phosphate, 1.7 Hz). In solution, addition of 0.5 mg/ml MPGM to 5mM 2,3-DPG (+ Mg) causes a 0.2 Hz shift (2-phosphate) which is increased to 2.3 Hz by 0.6 mg/ml phosphoglycerate kinase (PGK). These data are equivalent to a 1300 Hz (54 ppm) shift when the 2,3-DPG/MPGM/PGK complex is formed. Assuming 200 red cell surface ouabain sites (Hoffman, J. Gen. Physiol 54, 343, 1969; Perrone and Blostein, Biochim. Biophys. Acta 291, 680, 1973) each surface bound ouabain affects 2,3-DPG binding of about 10^3 molecules within the cell. This amplification factor could be much smaller if the cellular resonance shifts were much greater than that of the 2,3-DPG/MPGM/PGK complex or if the ouabain site number were increased. Our resonance shift is much larger than usually observed on ^{31}P binding and an increase even of an order of magnitude appears unlikely, as is also true of the ouabain site number. The evidence points to a cellular communication network by which one ouabain molecule at the cell surface communicates with many 2,3-DPG sites within the cell. Similar computations have been made with glyceraldehyde 3-phosphate (G3P) whose binding to glyceraldehyde 3-phosphate dehydrogenase on inside out red cell vesicles is also inhibited by ouabain. In this case a single molecule appears to be able to affect more than 10^4 G3P binding sites. (Supported in part by NIH grants 5 ROI GM15692-08 and 5 ROI HL14820-05)

W-AM-E4 SURFACE CHARGE DENSITY AND SIALIC ACID CONTENT OF DENSITY FRACTIONATED HUMAN ERYTHROCYTES. R.J. Knox, F.J. Nordt, D.H. Regan* and G.V.F. Seaman, Department of Neurology, University of Oregon Health Sciences Center, Portland, Oregon 97201.

It has been reported that decreases in red cell surface charge density accompany aging in vivo and that these alterations play a critical role in the recognition and elimination of effete red cells by macrophages in the reticuloendothelial system. We have attempted to reproduce these results and have examined the relationship between cell surface sialic acid and cell surface charge density for the extreme density fractions of fresh human erythrocytes. Contrary to the earlier reports we observe no significant difference in surface charge density for the extreme 5% fractions based on the electrophoretic behavior of the cells. However, small but significant decreases in the level of membrane-bound sialic acid are consistently observed in the densest red cell fractions. Sialic acid is the major contributor to red cell surface charge. There are also decreases in mean corpuscular volume and the levels of red cell glutamic-oxalacetic transaminase activity. The loss of membrane-associated sialic acid without change in the electrophoretic mobility of the red cells can be largely accounted for by the decrease in surface membrane area which accompanies loss of portions of the membrane during its long journey through the circulation. Data in the literature which indicates decreases in the total amount of many membrane components but a constancy in the ratios of components to one another lends support to this hypothesis. Supported by the National Institutes of Health, Grant HL 18284.

W-AM-E5 ELASTIC BEHAVIOR OF SENESCENT HUMAN ERYTHROCYTE MEMBRANES. B.D. Smith*, P.T. La Celle* and P.L. La Celle. Depts. Medicine and Rad. Biol. & Biophys., University of Rochester School of Medicine & Dentistry, Rochester, NY 14642.

The observed rigidity of senescent erythrocytes, due in part to their relative sphericity from fragmentation loss of membrane, could also result from a decrease of membrane elasticity, a membrane factor which could affect the cell's rheologic behavior. This study of old cell membranes investigated elasticity as a function of membrane tension and rate of force application; susceptibility to fragmentation failure at low force; and compared these parameters to "model" normal RBC membranes 1) made spherical or 2) having increased protein cross-linking, to determine whether changes in membrane elasticity and failure behavior are important concommitents of aging. Elasticity of "old" RBC separated by hypaque gradients was determined by observing 1) membrane extension in 1.5 μm pipettes at forces $2x10^2$-$6X10^5$ dyn/cm^2, 2) membrane tension for local membrane failure and 3) evidence of permanent deformation of old cell membranes and of normal membranes treated with gluteraldehyde. Forces up to 3X those for control cells were required to elastically extend old cell and gluteraldehyde-fixed membranes; the extent of membrane deformation/unit membrane tension was 20 to 35% that of controls or fragmentation-induced spherical cells. Old membranes fragmented at forces of 5.5 to $6.5X10^4$ dyn/cm^2; control values = 2.2 to $2.6X10^4$. Old cells developed permanently deformed membranes. These data indicate that rigidity of senescent cell's membrane is due not only to relative sphericity, but also that significant reduction of membrane elasticity similar to that observed in membrane having increased protein cross-linking also contributes. Loss of elasticity and a tendency to permanent or plastic deformation could be the result of age related increase of membrane protein interactions in erythrocyte senescence.

W-AM-E6 ELECTRIC FIELD EFFECTS ON ERYTHROCYTE MEMBRANES R.A. Hoffman* Biophysics and Instrumentation Group, Los Alamos Scientific Laboratory, University of California, Los Alamos, N.M. 87545 and S.F. Cleary*, Biophysics Department, Medical College of Virginia, Virginia Commonwealth University, Richmond, Va. 23298. Saline suspensions of erythrocytes were exposed to pulsed electric fields or microwave irradiations, and potassium and hemoglobin release and the osmotic fragility of the cell membranes were measured. The pulsed fields decayed exponentially with time constants (τ) of 0.43 - 6.0 μS and had maximum amplitudes (E_o) of 1.8 - 4.8 kV/cm. Average sample heating was negligible. Cells were exposed to 1-3,000 pulses. For fixed values of τ and E_o, the release of potassium required the fewest pulses. Additional pulses then caused an increase in osmotic fragility, and further exposure resulted in hemoglobin release. A single 6.0 μS, 4.8 kV/cm pulse produced 100% potassium release and no hemoglobin release,whereas 3,000 pulses with τ= 0.43 μS and E_o = 4.8 kV/cm produced 40% potassium release and no hemolysis. The strong dependence of these effects on τ is not due to the time required to charge the cell membrane capacitance since this process has a time constant < 0.01μS. Minimal values of τ and E_o required to produce potassium release were estimated to be approximately 0.4 μS and 1.8 kV/cm respectively. A somewhat different process appears to be responsible for the induction of potassium release than for induction of hemoglobin release. Human erythrocytes were more sensitive to the pulsed fields than were rabbit or dog erythrocytes. Exposure of rabbit erythrocytes to 3.0 GHz microwaves with a power density of 50 mW/cm^3 produced only a small increase in osmotic fragility which was attributed to heating of the cell suspension.

W-AM-E7 PORES CREATED BY A TRANSMEMBRANE POTENTIAL CAUSE HEMOLYSIS OF HUMAN ERYTHROCYTES. Kazuhiko Kinosita* and Tian Yow Tsong, Department of Physiological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

An electric pulse was applied to isotonic suspensions of human erythrocytes. This external electric field generates a potential difference across the cell membrane. The transient transmembrane potential that exceeds a threshold (about 1 V) causes hemolysis of the red cells. Kinetic measurements show that the hemolysis (i.e. release of hemoglobin) occurs only after a certain latency period. During this period the erythrocytes are permeable to sodium and potassium ions which normally do not penetrate the membrane in appreciable amount. These facts suggest the following mechanism for the hemolysis reaction. First the transmembrane potential creates or opens up in the membrane pores which allow the passage of small-sized molecules but not of hemoglobin. The equilibration of ions through these pores leads to the swelling of the erythrocytes, as has been confirmed by a hematocrit measurement, because of the osmotic pressure of hemoglobin inside. Once the cell volume reaches a limit, the erythrocytes "puncture" and release hemoglobin. The size of the field induced pores can be controlled by adjusting the ionic strength of the medium: pores formed in an isotonic saline are small and block sucrose molecules, while larger pores that admit sucrose are obtained by pulsation at lower ionic strengths. The pore size also depends on the magnitude and duration of the transmembrane potential. Presence of calcium reduces the pore size. Under certain conditions the pores can be resealed before the hemolysis takes place. (This work was supported by NIH Grant HL 18048).

W-AM-E8 CHOLINE FLUXES IN A MARINE PSEUDOMONAD: THE EFFECT OF MEMBRANE PERTURBERS. Anthony Verbalis,* Wallace Snipes and Alec D. Keith, Department of Biochemistry and Biophysics, The Pennsylvania State University, University Park, Pa. 16802

The bacterium Pseudomonas BAL-31 accumulates radioactivity from methyl-^{3}H choline to a steady state concentration ratio of 10^5, with a K_m for the initial rate of 7×10^{-7}M. Radioactivity is not incorporated into lipids or other macromolecules, but evidence is presented that choline is chemically modified after entering the cell. Both the transport protein and the modification enzyme are inducible, although lower levels are present constitutively. Under energy poor conditions (lack of O_2) uptake ceases and internal unmodified choline leaks out via a carrier mediated process, while the modified substrate leaks passively through the membrane barrier. The existence of these three fluxes (energy dependent uptake, facilitated efflux and passive efflux) offers an assay for the effects of membrane perturbations on the function of a membrane protein. In particular, it may be possible to decide whether the altered function is due to a change in state of the lipids or to a direct interaction between the carrier protein and perturbing molecule. Adamantanone, a spherical non-polar molecule with a polar ketone group, decreases influx, increases passive efflux and decreases facilitated efflux. Data concerning the perturbing effect of this and other molecules will be discussed.

W-AM-E9 CROSS-LINKING STUDIES OF THE Ca^{++}-Mg^{++} ATPASE PROTEIN. Ronald J. Baskin and Stephen D. Hanna,* Department of Zoology, University of California, Davis, California 95616

The addition of cupric phenanthroline, a cross-linking catalyst, to vesicles prepared from rabbit and lobster sarcoplasmic reticulum membranes caused protein sulfhydryl groups to form disulfide bridges. This reagent affected only the Ca^{++}-Mg^{++} ATPase protein. Other membrane proteins such as calsequestrin and calcium binding protein were unaffected. As a result of cross-linking, dimer, trimer and tetramer formation was observed with estimated molecular weights of 257,000 daltons, 351,000 daltons and 422,000 daltons respectively. Prolonged treatment (> 5 min) resulted in the formation of oligomers too large to enter the gel system. Cross-linking of the Ca^{++}-Mg^{++} ATPase protein has previously been carried out using dimethylsuberimidate (Louis and Shooter, Arch. Biochem. Biophys. 153, 641-655 (1972)). This reagent showed the serial formation of oligomers beginning with dimers. More recent studies using cupric phenanthroline (Murphy, BBRC, 70, 160-166 (1976)) showed the formation only of tetramers and multiples of tetramers. This is in contrast to the present studies which showed dimer and trimer formation. Freeze-fracture electron micrographs of vesicles subjected to prolonged treatment with cupric phenanthroline exhibited extensive aggregation of the intramembranous 80 Å particles. While the present results support indications that an oligomeric unit of the Ca^{++}-Mg^{++} ATPase protein may be responsible for Ca^{++} transport and exist in the membrane as a functional unit, they could simply reflect random cross-linking of monomeric units in a fluid membrane. (Supported in part by USPHS Research Grant #HL 12978-07.)

W-AM-E10 A LIGHT SCATTERING STUDY OF THE EFFECTS OF CALCIUM ION TRANSPORT ON SARCOPLASMIC RETICULUM VESICLE STRUCTURE. J.C. Selser, Y.Yeh, and R.J. Baskin, Departments of Applied Science (Y.Y. and J.C.S.) and Zoology, University of California, Davis, CA 95616.

Photon correlation spectroscopy (PCS) and electron microscopy (EM) were used to investigate osmotically driven changes in the structure of sarcoplasmic reticulum (SR) vesicles. While vesicles shrank uniformly for sucrose concentrations in the range 0.0M to approximately 0.7M, they became "rigid" from approximately 0.7M to 1.0M although EM results showed vesicles remained spherical and had not collapsed (Biophys. J., Dec., 1976). Upon active calcium transport at physiological concentration and in the absence of oxalate, however, vesicles already shrunken in sucrose solution shrank even further although no such effect was observed when calcium was introduced passively into an identical sucrose solution control nor was any change in PCS results observed upon addition of calcium to an isotonic aqueous control. These results suggest that negative charge repulsion may play a significant role in the observed rigidity of SR vesicles under osmotic stress and suggest further that the transport process is Ca^{++} specific since a relaxation of osmotic stress was not observed during or after transport. (This work supported in part by NSF grant PCM 73-06918-04 and NHLI grant HL12978-07)

W-AM-E11 CALCIUM BINDING TO GUINEA PIG CARDIAC SARCOLEMMA. A.L. Jacobson and C. Morcos*, Biochemistry Group, Chemistry Department, The University of Calgary, Calgary, Alberta, Canada, T2N 1N4.

Calcium binding to guinea pig cardiac sarcolemma has been measured (using $^{45}Ca^{+2}$) under a variety of conditions. The sarcolemmal preparations consisted of fragmented sheets and addition of inorganic phosphate did not increase the binding. Calcium is bound to the membranes both in the presence and absence of ATP. Schatchard plots showed that in the absence of ATP there are two classes of calcium binding sites. There is only one class of ATP dependent calcium binding sites. At high ATP concentrations approximately 80% of the calcium bound is ATP dependent. The increase in calcium binding on addition of ATP paralleled the measured ATPase activity. Inhibition of ATPase activity with sulfhydryl reagents produced a parallel inhibitor of calcium binding. Calcium binding is relatively fast. With 0.2 mM ATP approximately 60% of the calcium is bound within 30 seconds. With 5.0 mM ATP, the same amount of calcium was bound within 30 seconds but this represented only 30% of the total.

Sodium specifically inhibits calcium binding. Addition of sodium chloride decreased both the non-ATP dependent and the ATP dependent calcium binding. The decrease in the ATP dependent portion was greater than could be accounted for by the changes in the non-ATP dependent calcium binding. Potassium ions had no effect on calcium binding. The net effect when both sodium and potassium ions were present, was a decrease in the calcium binding. Addition of magnesium ions produced only a small (7%) increase in calcium binding.

W-AM-E12 A FLUORESCENCE ENHANCEMENT ASSAY OF CELL FUSION. Paul Malcolm Keller*, Stanley Person and Wallace Snipes. Laboratory of Biophysics, Department of Biochemistry and Biophysics, The Pennsylvania State University, University Park, PA 16802

Cell fusion induced by a syncytia-producing mutant of Herpes Simplex Virus type I was monitored by measuring the resonance energy transfer between two probes which consist of fluorescent molecules conjugated to saturated hydrocarbon chains to insure their localization into cellular membranes. Resonance energy transfer requires that the two probes be close together and that there be an overlap of the emission spectrum of the donor probe and the absorption spectrum of the acceptor probe. At appropriate wavelengths resonance energy transfer can be measured by preferentially exciting the donor probe and observing the fluorescence of the acceptor probe. Two cell populations, each labeled with one of the probes, were infected with virus, mixed in equal proportions, and the fluorescence of the acceptor probe measured as a function of time after infection. An increase in fluorescence was observed beginning at the time of onset of cell fusion. An investigation of the distance dependence indicated that the increase in fluorescence was mainly due to resonance energy transfer and not to photon emission and reabsorption. The possible application of this assay to other types of membrane fusion is noted.

W-AM-E13 ON MACULA COMMUNICANS STRUCTURE. G. Zampighi and J.D. Robertson, Dept. of Anatomy Duke University, Durham, NC.

The macula communicans (gap junction) is a junctional specialization of contacting cell membranes which seems to mediate electrotonic coupling. En face views show a hexagonal array of particles spaced ∿ 8.5 nm apart. Some believe that each particle extends through both membranes and contains an ∿ 2.5 nm acqueous channel. It seems reasonable to predict that such channels exposed to phosphotungstic acid (PTA), would fill and show up as dense transverse striations spaced at 8.5 nm intervals in favorably oriented specimens. However, high resolution electron microscopy has failed to demonstrate convincingly the predicted striations. In order to study this problem further 100g. of liver was homogenized in an isotonic medium containing 5μm of phenylmethyl sulfonyl fluoride (an antiproteolytic agent). A bile canaliculum (BC) fraction was separated by differential and sucrose density gradient centrifugation. The BC fraction was partially solubilized with sodium deoxycholate (DOC) at a ratio of 5mg DOC/mg protein and centrifuged at 25,000g. The pellet was further separated into components by removing DOC by means of treatment with a detergent, Lubrol WX. The final separation of the pure junctional fraction was accomplished by sucrose density gradient centrifugation with suitable control of pH and ionic strength. This treatment allows the separation of the junctions without use of proteolytic enzymes. By morphological criteria, the fraction was 90% pure. The main contaminants were collagen fibers and amorphous material. SDS-polyacrylimide gel electrophoresis showed one main protein band at 25,000 daltons. This procedure yields junctions with sufficiently high long range order in the hexagonal array to permit an analysis by the method of Unwin and Henderson (J.Mol.Biol. 94: 425, 1975). The fraction is being studied now by this method as well as other physical and chemical techniques.

W-AM-E14 EFFECTS OF BUTYLATED HYDROXYTOLUENE (BHT) ON HERPES SIMPLEX VIRUS. Paul Wanda*, Tom Holland*, Paul Keller*, Stanley Person, Alec Keith, and Wallace Snipes, Biophysics Program, The Pennsylvania State University, University Park, PA 16802

Most antiviral agents that are currently being developed are designed to interfere with viral nucleic acid synthesis. An alternate and complementary approach for the control of enveloped viruses may be the use of hydrophobic, membrane-perturbing molecules that interfere with viral membrane functions. One such molecule, butylated hydroxytoluene (BHT), is a potent inactivator of Herpes Simplex Virus (HSV) in vitro under conditions that are comparatively harmless to the host cells. Sucrose gradient analysis of radioactively labeled virus shows that the viral envelope is not removed during treatment with BHT. Furthermore, inactivated virions are capable of attaching to the host cells. In addition to the contact inactivation of HSV, BHT may interfere with other virus-associated membrane processes. As an example, cell fusion caused by syncytia-forming mutants of HSV is markedly reduced by BHT. Studies are underway to determine whether BHT primarily affects membrane lipids, membrane proteins, or lipid-protein interactions.

W-AM-F1 COUPLING OF LIGHT-INDUCED CATION GRADIENTS TO AMINO ACID TRANSPORT IN HALOBACTERIUM HALOBIUM. J. K.Lanyi and R. E. MacDonald*, NASA-Ames Research Center, Moffett Field, CA, 94035, and Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY, 14850.

Illumination causes the extrusion of protons from bacteriorhodopsin-containing cell envelope vesicles, prepared from H. halobium. The resulting protonmotive force (ΔpH, interior alkaline and $\Delta\Psi$, interior negative) is accompanied by the extrusion of Na^+. The kinetics of external pH changes are greatly influenced by the amount of Na^+ inside the vesicles and suggest the operation of electrogenic H^+/Na^+ antiport ($H^+/Na^+ > 1$). The Na^+ gradient ($Na^+_{out}/Na^+_{in} >> 1$) which develops during illumination will drive the active transport of nineteen amino acids. Transport in every case can also be energized by an artificially created Na^+ gradient in the dark. Glutamate transport appears to be indifferent to the electrical potential developed, and this amino acid is probably translocated with Na^+ in an electrically neutral fashion. All the other amino acids are transported in response to electrical potential even in the absence of a Na^+ gradient, but all require Na^+ on the vesicle exterior. These amino acids thus appear to be transported electrogenically, and probably together with Na^+. Systems which symport amino acids with H^+ are not excluded in this organism, but if existing they would still require Na^+. The participation of Na^+ in energy coupling in halophiles is obviously related to their adaptation to a saline environment, and provides an example of procaryotic Na^+-driven transport. This kind of energy coupling had previously been thought to exist only in eucaryotes.

W-AM-F2 INHIBITION OF LIGHT-INDUCED METHIONINE TRANSPORT IN HALOBACTERIUM HALOBIUM VESICLES BY CYSTEINE. S. L. Helgerson and J. K. Lanyi, Extraterrestrial Biology Division, NASA-Ames Research Center, Moffett Field, CA 94035.

Illumination of H. halobium cell envelope vesicles containing bacteriorhodopsin results in the development of a transmembrane protonmotive force. Methionine is actively transported into the vesicles, primarily in response to the electrical potential component of the total driving force. Cysteine inhibits the uptake of methionine without dissipating the driving force since the transport of glutamate is not affected. The K_m for methionine transport (5.7μM) is unchanged in the presence of 30μM cysteine (5.0μM) indicating noncompetitive inhibition; the K_i of cysteine inhibition is 30μM. Glutathione causes similar inhibition. Cystine (400μM) does not inhibit methionine transport and has no affect on the degree of inhibition by cysteine or glutathione. Dithioerythritol and mercaptoethanol at a concentration of 1mM inhibit methionine transport by only 15-20%. The inhibition of methionine transport by cysteine can be completely reversed by washing the vesicles twice in 3M salt; reversal was not obtained when air was bubbled through the reaction mixture for 30 min. The effects of sulfhydryl reagents and of other oxidizing and reducing reagents on methionine transport are being studied in order to define the mechanism of inhibition by cysteine. Since cysteine uncouples the transport of methionine from the transmembrane protonmotive force, an understanding of this inhibition will provide evidence for the mechanism of energy coupling between the driving force and the amino acid carrier protein within the membrane.

W-AM-F3 FURTHER STUDIES OF MEMBRANE POTENTIALS AND RESISTANCES OF GIANT MITOCHONDRIA AND MITOPLASTS; METABOLIC DEPENDENCE AND VIABILITY. Bruce L. Maloff, Stylianos P. Scordilis, and Henry Tedeschi, The University at Albany, Albany, N.Y. 12222.

Direct measurement with microelectrodes indicates that the mitochondrial membrane potential shows no direct metabolic dependence. Liver mitochondria from cuprizone treated mice with diameters from about 5-10 μm were used. The microelectrode tips were in the inner mitochondrial space since (a) the potentials in the presence of valinomycin depended on the K^+ concentration of the medium and the magnitude of the K^+ diffusion potentials were consistent with the presence of a high internal concentration of K^+; (b) similar results were obtained with mitochondria from which outer membranes had been removed, and (c) punch-through experiments, in which the microelectrode traversed the mitochondria showed a single membrane potential in the presence or absence of valinomycin. We have developed assays for the metabolic viability of single impaled mitochondria which include the massive accumulation of calcium phosphate and the contraction of myofibrils isolated from glycerinated rabbit psoas muscle. Preliminary experiments indicate that the impaled mitochondria are viable. Aided by NIH grant NS07681. We wish to thank Charles Bowman, Christopher Reynolds and Kathleen Walsh Kinnally for their assistance in the mitoplast experiments.

W-AM-F4 ELECTROFLUORIMETRIC ESTIMATES OF MEMBRANE POTENTIAL IN METABOLIZING MITOCHONDRIA. Kathleen Walsh Kinnally and Henry Tedeschi, The University at Albany, Albany, N.Y. 12222.

A number of anionic and cationic fluorescent dyes have been investigated as possible monitors of the membrane potential of rat liver mitochondria or giant mitochondria from the liver of mice maintained on a diet containing cuprizone. The fluorescence of four dyes (ANS, 8-anilino-1-naphthalene sulfonic acid; diS-C_3-(5), 3,3'dipropylthiocarbocyanine iodide; diBa-C_4-(5), bis [1,3-dibutylbarbituric acid-(5)]-pentamethine oxonol; MI, merocyanine 540) was found to respond appropriately to changes in valinomycin induced K^+ diffusion potentials. The fluorescence of at least two of the dyes in suspensions of giant mitochondria varies linearly with the membrane potential measured directly with microelectrodes. Valinomycin induced K^+ diffusion potentials and occasionally potentials measured with microelectrodes were used to calibrate the dependence of the fluorescence on the membrane potentials. Upon the addition of substrate, the apparent potential change was variable with each of the dyes used, but generally less than -50 mv. The fluorescence of at least some of the dyes is thought to depend upon their concentration [J.F. Hoffman and P.C. Laris, J. Physiol. 239, 519 (1974)]. Since the distribution of anions and cations may be affected by phenomena unrelated to membrane potentials [H. Tedeschi, FEBS Lett. 59, 1 (1975)], these values need not reflect membrane potentials. While ANS and MI were found to have no metabolic effect, diBa-C_4-(5) and diS-C_3-(5) inhibited succinate and β-hydroxybutyrate oxidation respectively. DiS-C_3-(5) had no effect on succinate oxidation but inhibited phosphorylation. Aided by NIH grant NS07681. We thank A. Waggoner for a generous supply of dyes.

W-AM-F5 AMINO ACID INCORPORATION AND PROTEIN SYNTHESIS IN *EUGLENA GRACILIS* DURING ADAPTATION TO GROWTH IN THE PRESENCE OF DINITROPHENOL. J.S. Kahn and R.T. McConnell*, Biochemistry Department, North Carolina State University, Raleigh, North Carolina 27607

Euglena gracilis form giant mitochondria during adaptation to growth in the presence of uncouplers of oxidative phosphorylation. We investigated the synthesis of protein in different cell fractions during the process of adaptation, with special emphasis on changes in mitochondrial membrane proteins. Our data indicate that the formation of giant mitochondria is accomplished by fusion of existing ones, and does not involve any major *de novo* synthesis of mitochondrial membrane proteins. When mitochondrial membranes from cells pulse-labeled with ^{3}H-phenylalanine during adaptation were resolved by electrophoresis in SDS-polyacrylamide gels as well as by electrofocusing, no uniquely labeled peptide could be detected. Moreover, the relative incorporation of label into mitochondrial membranes relative to other cellular fractions showed an actual decrease during the process of adaptation. In an attempt to estimate protein turnover in normal vs. adapted cells, starved cells were pulse-labeled and then fractionated as above. It was found that while normal cells incorporate appreciable amounts of the labeled phenylalanine, adapted ones incorporated only 2-5% as much even when growing vigorously. The latter also showed only traces of label in the TCA-soluble pool. Since even in normal cells the incorporation of exogenously supplied, labeled, phenylalanine represented only a small fraction of the total phenylalanine incorporated into newly synthesized protein, it was concluded that the mechanism for phenylalanine transport was inhibited in the uncoupler-adapted cells thus curtailing the incorporation of the labeled amino acid.

W-AM-F6 MECHANISM OF CITRATE TRANSLOCATION THROUGH THE INNER MITOCHONDRIAL MEMBRANE. E. Kun, E. Kirsten* and M.L. Sharma*, Depts. of Pharmacology, Biochemistry and Biophysics, and the Cardiovascular Research Institute, University of California, School of Medicine, San Francisco Medical Center, San Francisco, California 94143.

Kinetics of citrate influx and efflux through the inner mitochondrial membrane were determined in phosphorylating, lysosome-free mitochondria operating as an open system resembling intracellular environment. The non-penetrating substrate analog, (-)erythrofluoromalate, was an obligatory cofactor to induce citrate influx, analogous to the physiological activator L-malate. Binding of ^{14}C(-)erythrofluorocitrate at a concentration of 30 to 50 pmoles per mg mitochondrial protein specifically and irreversibly inhibits bidirectional citrate flux. It was found that isolated inner mitochondrial membrane vesicles covalently bind both citrate and fluorocitrate, indicating different kinetics and distinct sites. The bound citrate is released by 0.2 M neutral hydroxylamine, implying the participation of macromolecular thioester bond. Thiol binding reagents and Fe^{3+} complexing agents and CN^- inhibit citrate and F-citrate binding to the inner membrane vesicles. Thioester bond between membrane associated -SH and F-citrate is much more stable than with citrate, explaining the irreversible inhibition and the toxicity of F-citrate. A catalytic model of energy-coupled citrate translocation is proposed involving a macromolecular thioester intermediate.
Supported by NIH grants GM-20552 and HL-6285.

W-AM-F7 MAGNESIUM ION INDUCED CONTRACTION OF LIVER MITOCHONDRIA. P. V. Blair, Department of Biochemistry, Indiana University School of Medicine, Indianapolis, Indiana 46202

The addition of 1 mM $MgCl_2$ to partially swollen rat liver mitochondria respiring in an isoosmotic sucrose-sodium phosphate or sodium acetate medium containing 1 mM EDTA and 15 mM succinate (pH 7.2) initiates contraction, inhibits respiration, and alters the ultrastructural configuration of the inner membrane-cristae-matrix continuum. The maximal extent of contraction attainable with $MgCl_2$ in the phosphate medium is about 80% of the maximal contraction induced by 2,4-dinitrophenol or anaerobiosis, but exceeds the maximal contraction induced with ADP by about 10%. The extent of mitochondrial contraction and the inhibition of succinate oxidation are dependent on $MgCl_2$ concentration. 1,000 µM $MgCl_2$ immediately inhibits about 70% of the succinate oxidation and initiates maximal extent of contraction concomitant with a distinct configurational change in ultrastructure which appears to differ from that initiated by either ADP or dinitrophenol. 150 µM $MgCl_2$ does not inhibit the rate of succinate oxidation but it does initiate about 75% of the maximal contraction attained with 1,000 µM $MgCl_2$. The rate of mitochondrial contraction is dependent on both the phosphate and $MgCl_2$ concentration. $CaCl_2$, in contrast with $MgCl_2$, immediately stimulates succinate oxidation and after a sharp contraction spike of short duration initiates additional expansion of the inner membrane-cristae-matrix continuum. The contraction spike, induced by $CaCl_2$ occurs in a reaction system containing either phosphate or acetate. The results are consistent with the notion that Mg^{2+} and Ca^{2+} may modulate mitochondrial volume and exert a degree of control over certain oxidative processes. (Supported by a grant from the Grace M. Showalter Residuary Trust.)

W-AM-F8 CA^{++} TRANSPORT BY MITOCHONDRIA IN SITU. A. Scarpa, F. J. Brinley, Jr. and T. Tiffert, Dept. Biochemistry and Biophysics, Univ. Pennsylvania, Philadelphia, Pa. 19174 and Dept. Physiology, Univ. Maryland, Baltimore, Md. 21201.

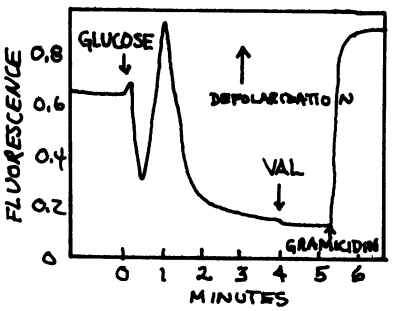
A non-destructive technique was developed for measuring ionized Ca^{++} concentrations in the cytosol of giant cells and the changes of $[Ca^{++}]$ due to metabolic and electric events. The technique is based upon the introduction in the cytosol of an indicator sensitive to ionized Ca^{++} and the kinetic detection of the indicator absorbance in situ by sensitive pulsed multiwavelength microspectrophotometry. The Ca^{++} indicator arsenazo III was microinjected (200-600 µM) in squid giant axons which were dissected from living specimens of Loligo Pealei and positioned in chambers containing artificial seawater. The addition of an uncoupler of oxidative phosphorylation induced an increase of the ionized Ca^{++} from 50 nM, corresponding to the resting level, to about 200 nM. Due to the limited volume occupied by the mitochondria (1/100) and the high Ca^{++} buffering capacity of the axoplasm, this indicates that large amounts of Ca^{++} are present within mitochondria in situ. During electrical stimulation of the axon, which causes a movement of Ca^{++} from the seawater to the axoplasm, the axoplasmic ionized Ca^{++} increased linearly to 30-50 µM, when the axon was pretreated with uncoupler. In the absence of uncouplers, the ionized Ca^{++} increased during the stimulation only to 3-8 µM to remain constant at that level, in spite of the large amount of Ca^{++} entering the axoplasm. The Ca^{++} accumulated by the mitochondria under these conditions is totally and rapidly released by uncouplers or CN^- in the presence of apyrase or valinomycin. Under high Ca^{++} loading, the uptake of Ca^{++} by mitochondria in vivo was more effective with coupled respiration alone than with ATP hydrolysis alone. These results obtained in vivo permit us to evaluate rates, capacity and affinity of mitochondrial Ca^{++} transport obtained in vitro and to assess the various mechanisms proposed for Ca^{++} uptake.

W-AM-F9 DIRECT INTRAMITOCHONDRIAL LACTATE OXIDATION BY RABBIT SPERM MITOCHONDRIA. B.T. Storey and F.J. Kayne*, Depts. of Obstetrics & Gynecology, Biochemistry & Biophysics, and Physiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19174.

Suspensions of hypotonically treated rabbit epididymal spermatozoa (HTRES), in which the mitochondria are fully intact and accessible to added reagents, oxidize added lactate rapidly in the presence of added NADH. The active extramitochondrial lactate dehydrogenase (LDH) bound to the cell structure and accessible to added NADH (Storey & Kayne [1975] Fertil. Steril. 26:1257) ensures that any pyruvate, either present as impurity or formed extramitochondrially, is rapidly reduced. Lactate oxidation is partially inhibited by pyruvate and completely inhibited by rotenone. Fluorimetric studies of the reduction of intramitochondrial NAD+ showed that malate plus lactate reduce the same pool of this component as do malate plus acetyl carnitine. Both sets of substrates give the same maximal degree of reduction in the presence of rotenone; the reduced intramitochondrial NADH is reoxidized by subsequent addition of pyruvate. Lactate reduction of intramitochondrial NAD+ in the aerobic steady state with ADP (State 3) is reversed by added pyruvate which also inhibits O_2 uptake; subsequent addition of malate restores both NAD+ reduction and O_2 uptake. These results show clearly that the mitochondria of rabbit sperm contain LDH which utilizes matrix NAD+ as coenzyme. LDH activity released into the suspending medium by sonication of HTRES was shown to behave as LDH-X on gel electrophoresis. Over 90% of the total LDH activity is functionally extramitochondrial, but the intramitochondrial LDH is sufficiently active that rabbit sperm can utilize lactate as a primary energy source and a pyruvate/lactate shuttle to reoxidize cytosolic NADH generated by glycolysis. (Supported by USPHS HD-06274 and K04-GM-70249 and NSF PCM-74-12358).

W-AM-F10 KINETICS OF MEMBRANE POTENTIAL CHANGES IN *S. LACTIS* CELLS FOLLOWING ADDITION OF GLUCOSE. D. P. Carbone* and A. S. Waggoner, Department of Chemistry, Amherst College, Amherst, Mass. 01002

Changes in the transmembrane electrical potential of energy-starved *S. lactis* cells following glucose addition were measured by the use of two fluorescent dyes, merocyanine 540-C_3 and diS-C_2-(5). The fluorescence response for a suspension of cells in Na phosphate buffer, pH 7.5, containing diS-C_2-(5) is shown below. The "rebound" following glucose addition is also reflected in the intracellular ATP concentration (firefly luciferase assay) which is linked energetically to the membrane potential and pH gradient by a membrane bound (H^+)-ATPase. The fluorescence rebound is absent if the suspension medium pH is lowered to 5.5 or if choline is used in place of Na or K in the suspension medium. The relative merits of several possible mechanisms for the rebound (including competition for ATP between the early "feedback" steps of glycolysis and the (H^+)-ATPase, and allosteric switching of permeases by nucleotide phosphates and pH changes) will be discussed.



W-AM-F11 THE RATE OF QUATERNARY STRUCTURE CHANGE IN HEMOGLOBIN MEASURED BY MODULATED EXCITATION. F.A. Ferrone, Laboratory of Chemical Physics, NIH, Bethesda, Md. 20014 and J.J. Hopfield, Department of Physics, Princeton University, Princeton, N.J. 08540 and Bell Telephone Laboratories, Murray Hill, N.J. 07974.

We have used a new technique, employing the modulated photodissociation of carbon monoxide from hemoglobin, to obtain the rates for conversion between the two quaternary states, R and T at three-fold ligation. We obtain values of 780 ± 40 sec^{-1} for R to T, and a rate of 2500 ± 200 sec^{-1} going from T to R. (These are at 22°C and pH 7.) From these rates we find an equilibrium constant L_3 of 0.31 ± 0.04. This value of L_3 agrees well with previous estimates, and the degree of agreement between our measurement and the value predicted by the allosteric model provides a quantitative test of that model. In contrast, a sequential model for the structural change was found incompatible with our data, even if kinetic inequivalence of the α and β chains was allowed.

The technique, which will also be described, can be applied to any system that can be repetitively excited, and in which the response scales linearly with the excitation.